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Studies of Vetch Anthracnoses.

Norman Louis Horn
Louisiana State University and Agricultural & Mechanical College

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Norman Louis Horn, Jr.
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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

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ABSTRACT

A species of Colletotrichum was consistently associated with and isolated from diseased common vetch, Vicia sativa, collected from several different areas in Louisiana during the late winter and spring months of 1948, 1949, and 1950. The anthracnose was more severe in southern Louisiana, where the plants were completely defoliated and killed. The first symptoms of the disease occurred as leaf and stem spots, both of which became sufficiently numerous to cause leaf blight and stem blackening. A detailed study was made of this fungus, isolate 192 type, and C. villosum Weimer, isolate 303 type. In greenhouse inoculation tests, common vetch plants were killed within seven days by isolate 192 type. On the basis of greenhouse pathogenicity tests V. villosa, V. atropurpurea and V. ludoviciana were resistant to isolate 192 type. V. sativa, V. dasycarpa, V. angustifolia, V. pannonica, V. grandiflora, V. articulata, V. hirsuta, V. alba, and V. aurantia were susceptible. V. alba, V. sativa, V. angustifolia, V. pannonica, V. grandiflora, V. aurantia, and V. hirsuta were resistant to C. villosum, isolate 303. The other vetch species tested were susceptible. Austrian Winter peas (Pisum sativum var. arvense) and Dixie Wonder peas (P. sativum var. arvense) and Creole peas (P. sativum) were susceptible to isolate 192 type in greenhouse tests, as well as in nursery plots adjacent to severely diseased common vetch. Singletary peas (Lathyrus hirsutum), and sweet peas (L. odoratus), and Melilotus indica were susceptible to the same fungus. The same varieties of these three plant species were not susceptible to C. villosum (isolate 303). On the basis of pathogenicity tests and morphological, physiological and cultural studies the isolate 192

type is believed to be a species of Colletotrichum different from any reported attacking vetch.

INTRODUCTION

Among the leguminous plants recommended in Louisiana (8, 10, 17, 26, 27) for growing during the colder months of the year, the vetches (*Vicia* spp.) have been widely used for soil improvement. *Vicia sativa*, common vetch, and *V. villosa* Roth., hairy vetch, have been more widely used than the other species. Agronomists in Louisiana have recommended the fall planting of winter legumes to be plowed under in the spring as a source of nitrogen and organic matter. Cotton, the most common crop used in conjunction with winter legume tests, gave increased yields following a winter crop of vetch and also peas (7, 8). Certain agronomic practices were found to be essential for the maximum growth of these winter cover crops (8). Among these are, inoculation of the seed with *Rhizobium* spp., adequate drainage, and application of phosphate fertilizers. Even though agronomic recommendations have been followed, many farmers have not been very successful in growing leguminous cover crops. Observation and preliminary studies indicated that diseases were in a large part responsible for the poor growth and early dying of the vetches, particularly common vetch. Disease surveys and complaints from growers received through county agents indicated that the winter legumes were more adversely affected by diseases in south and central Louisiana than in the northern parishes.

Preliminary studies showed that fungi belonging to the genus *Colletotrichum* were chiefly associated with diseased common and hairy vetch plants. Among the fungi reported in the literature as attacking vetch were *C. villosum* Weimer and *C. viciae* Dearn. and Overh. As preliminary studies showed that the *Colletotrichum* found attacking

vetch in Louisiana differed from Colletotrichum villosum and C. viciae in certain respects, an extensive study of this fungus was carried out. These studies, together with studies on certain other anthracnose fungi found associated with diseased vetch, constitute the work being presented as a thesis. Preliminary reports have been published (15, 16).

LITERATURE REVIEW

The reports in the literature dealing with the anthracnose fungi on Vicia spp., excluding V. faba, are concerned with four Gloeosporium species. These include G. viciae Pautrey and Roum (22), a form described in 1890 which occurred on stems of V. cracca in France; G. davisii E. and E. (24) and G. Everhartii Sacc. and Syd., first described as G. americanum E. and E. (24), occurred on the leaves of V. americana in the United States; and G. tricolor Lind. (23), which was reported to cause "frog-eye" leaf spot of V. cracca in Denmark in 1907. The first three species produce smaller conidia than Colletotrichum villosum and the undescribed species of Colletotrichum (isolate 192 type). The latter species, G. tricolor, possesses much longer conidia than either of the two Colletotrichum species.

Colletotrichum villosum has certain features similar to Kabatiella nigricans (Atk. and Edg.) Karak. (34), previously described as Protocoronospora nigricans Atk. and Edg. (2). Under certain conditions K. nigricans produces stem and leaf spots similar to those caused by C. villosum. The conidia of these two fungi are essentially the same shape but those of C. villosum are somewhat longer than the conidia of K. nigricans. The method of conidial production and germination of the C. villosum are greatly different from those of K. nigricans. The former produces conidia singly on slender conidiophores which are nearly the same width as the conidia at the point of attachment. The latter produces numerous conidia on a much enlarged conidiophore; conidia bud on germination.

In 1919 (13) a Colletotrichum species was reported to occur on vetch in Alabama and in 1926 (14) on vetch in Louisiana. No description, however, of the fungus or the disease was given in either case. Dearness (9) in 1928 described C. viciae, which C. villosum resembles more than any other previously described species. C. viciae, however, does not produce definite leaf spots. Part or all of the leaflet is involved, the infected areas often containing scattered acervuli. The setae are much shorter, are more narrow at the base, and are continuous or have one septum.

Colletotrichum villosum was described in 1945 by Weimer (29). A later account of his publication was given in 1950 (18). He first reported the disease in 1941 (28) which he attributed to a Colletotrichum sp. and which presumably was caused by the same fungus. Several years later Person (19) called attention to a Colletotrichum species attacking hairy vetch in Louisiana. Reports by Bain (3, 4) in 1944 designated that C. viciae occurred on the stems and leaves of hairy vetch grown in Mississippi. He indicated in another report (5) the same year that a Colletotrichum species different from C. viciae was associated with diseased hairy vetch in Louisiana. He pointed out that in a 15 to 20 acre vetch planting near Ville Platte, Evangeline Parish, Louisiana, 40 per cent of the plants were killed and the remainder contained numerous leaf and stem spots probably caused by C. viciae. Bain (6) in 1945, however, reported that the Colletotrichum species which he mentioned previously to be associated with diseased vetch was not C. viciae but more like the type described by Weimer. In the same report Bain stated that C. villosum was found on Austrian Winter peas in Louisiana and Mississippi. In Alabama, Stone (25) reported blackstem

to be prevelent on vetch plants, often extending one half to two thirds up the stems. He attributed the disease to Colletotrichum spp., Ascochyta spp., and Kabatiella nigricans.

Reports indicate that C. villosum was found in Alabama, Florida, Georgia, Louisiana, and Oklahoma (20, 33). They suggest that hairy vetch was susceptible to this fungus while common vetch was resistant. Weimer (29, 30) stated that most selections of common vetch were resistant, according to greenhouse inoculation tests and field observations. The following year in 1946 (31, 32) he stated that anthracnose (C. villosum) was prevalent and destructive to common vetch; many pods were badly affected, and large areas of the stem and leaf tissue were involved. Strains of common vetch were particularly subject to anthracnose (C. villosum), as indicated by the Kentucky Experiment Station (1) the same year. Gilman and Tiffany (11) isolated C. villosum from vetch plants in Iowa.

Preliminary reports by Horn and Atkins (15, 16) indicated that a Colletotrichum species which resembled C. villosum in some respects caused considerable damage to common vetch in Louisiana in the spring of 1948, 1949, and 1950. Since this fungus differed from C. villosum in cultural, physiological, and morphological characteristics and in pathogenicity tests, it was considered a different species.

MATERIALS AND METHODS

Most of the commercial seed lots were obtained from the Louisiana Agricultural Co-Operative, Incorporated, Baton Rouge, Louisiana. These included common and hairy vetch, Austrian Winter peas, and Singletary peas. Dixie Wonder peas and Texas hairy vetch seeds were obtained from the Kalmback-Burckett Company, Incorporated, Shreveport, Louisiana. Sweet and Creole pea seeds were purchased from local seed stores. Other seed lots were furnished by the Agronomy Department, Louisiana State University. Seed lots with plant introduction and forage crop numbers were obtained from the following sources: Seeds with F. C. numbers were supplied by P. R. Henson, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Maryland. Seeds with P. I. numbers were supplied by H. L. Hyland, Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Maryland. Seed lot P. I. 190, 252 was the only one tested which was furnished by Dr. Edwin James, Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils and Agricultural Engineering, Experiment, Georgia. Seed lots 38, 41, and 42 were collected locally.

All plants were grown in Sharky soil sterilized at 15 pounds pressure for two hours in three-inch pots unless otherwise stated. The soil was used a week or more after this treatment.

Inoculations that were made with conidial suspensions were applied under very low air pressure with a specially prepared fog-type nozzle made of glass tubing. Sterilization between changes of inoculum was

accomplished by running alcohol through the nozzle for a short time, followed by a water rinse. Inoculated plants always were placed in the moisture chamber for three days. This chamber consisted of a wooden, framework structure covered with one thickness of muslin. The moisture was supplied with two fog-type nozzles similar to that described above. The cloth covering was wet continually during the time of incubation. The fogging apparatus was run only when necessary so that water did not run off the plants and yet the humidity in the chamber was at a maximum. Plants removed from this chamber were placed on a greenhouse bench or outside the greenhouse.

Isolations from infected field plants or from greenhouse inoculated plants were obtained from young spots on leaves and stems. The diseased tissues were cut into portions about one cm. square, immersed in diluted clorox (one part clorox to four parts distilled water) for two to three minutes, and plated on potato-dextrose agar. Transfers were made to potato-dextrose agar slants from the fungi that developed from the diseased plant tissue.

For want of better terms the following were used throughout this paper to designate conidial types: "straight", meaning nearly straight; falcate, meaning extremely curved; and curved, meaning conidia which were intermediate to the first two types.

Potato-dextrose agar was used as the standard culture medium and contained the following materials per liter unless otherwise specified: extracted juice from 200 grams of potatoes, 15 grams of agar, and 20 grams of dextrose.

FIELD SYMPTOMS AND LABORATORY EXAMINATION OF DISEASED PLANTS

During the spring of 1949 common vetch, Vicia sativa, and hairy vetch, V. villosa, plants grown on the Louisiana State University Horticulture farm, contained many leaf and stem spots. The latter spots were usually circular to elliptical and up to three mm. in diameter. They varied in color according to their age, the most recently formed ones being pale green in color as contrasted to the older ones which were light brown to gray, bordered by a narrow, reddish-brown band. In the center of the older spots sometimes was found a darkened spot which was later observed to be masses of acervuli. The stem spots varied in size and color. Some were similar in color and shape to those on the leaves. Others were more or less darkened streaks. They were slightly sunken, elliptical to much elongated, and often coalesced so that they girdled the stems or petioles. Individual spots ranged in size from less than a mm. wide and two mm. long to two mm. wide and five mm. long.

Diseased plant material was collected and brought into the laboratory for examination. Some was surface disinfected and placed in moisture chambers. Mounts were made from the stem and leaf spots of both common and hairy vetch. It was found that two distinct conidial types of Colletotrichum were involved, a curved conidial type and a "straight" conidial type. It was noticed further that the curved conidia were obtained most frequently from spots on hairy vetch and the "straight" conidia most frequently from the spots on common vetch. These facts suggested that two species of Colletotrichum existed and

that hairy vetch was more susceptible to the curved type while common vetch was more susceptible to the "straight" type.

Leaf spots produced by natural infection in the field were identical so that it was not possible to separate the two types of anthracnose on plant response alone. Since the spots were similar, the only means of identification was by microscopic examination of the conidia involved in infected tissue. It was found, however, that the "straight" conidial type was associated mostly with spots on common vetch leaves, whereas the curved conidial type was associated usually with spots on hairy vetch leaves (Plate I). Leaf and stem spots caused by the latter appeared early in March and became increasingly more numerous as the spring season progressed. Spots caused by the former type were first isolated in the middle of February and became more pronounced with the increasingly warmer temperatures.

Pod spots were not observed on greenhouse grown plants. However, spots were observed on pods of field grown plants (Plate II). Those found on hairy vetch pods were different in shape from those on common vetch pods. The former were up to four mm. long, one or two mm. wide, rather pointed at the ends, Claret-brown of Ridgway (21) to black, with a small nearly white center. Spore masses were found frequently in the center of these infection centers. The spots always were produced at an angle to the long axis of the pod, probably due to the angular formation of the pod cells. Spots on common vetch pods were very similar to leaf spots in shape and color, but were deeply sunken. They varied from less than one mm. to three mm. in diameter, were cinnamon brown to darker brown throughout, surrounded by a characteristic, narrow, Garnet-brown of Ridgway border. Acervuli, also, were produced from these infected areas.

From some of the diseased tissue placed in the moisture chambers two other conidial types of anthracnose were observed. One of these was falcate and pointed and the other straight and short with rounded ends.

Diseased plant material was collected from vetch plants in other parts of Louisiana. These same types of conidia were observed on diseased common and hairy vetch from St. Joseph, Louisiana, and were isolated from vetch plants collected at Zachary, Abbeville, and Winnsboro, Louisiana.

Observations were made of diseased field peas (Pisum sativum var. arvense), including Dixie Wonder peas and Austrian Winter peas obtained from the Louisiana State University Horticulture farm. These plants contained spots on stems and leaves which were circular to oval in shape and dark green in color. Diseased plant material was placed in a moisture chamber for several days and upon observation it was found that some of the fungi present produced conidia resembling several of the isolates obtained from vetch.

ISOLATIONS AND SOURCE OF ISOLATE STUDIED

Common and hairy vetch plants were grown for inoculation work as described in materials and methods. The following isolates were tested for pathogenicity in the first series:

Isolate 191 - "straight" conidial type Colletotrichum isolated from common vetch.

Isolate 192 - "straight" conidial type Colletotrichum isolated from common vetch.

Isolate 186 - "straight" conidial type Colletotrichum isolated from common vetch.

Isolate 193 - "straight" conidial type Colletotrichum isolated from field peas.

Isolate 303 - curved conidial type Colletotrichum isolated from vetch.

The inoculum was prepared by making conidial suspensions of the fungi grown on sterilized fresh string bean pods. Uninoculated plants were used as controls.

Spots appeared on some of the plants four days after inoculation and three days later the degree of infection was recorded. Symptoms were similar in most respects to those observed on field grown plants. Reisolations were made from these inoculated plants. Table 1 indicates the frequency of reisolation of these isolates.

Table 1.--The frequency of reisolation from artifically inoculated, greenhouse-grown vetch plants.

Isolate No.	No. isolations made	No. reisolated	No. sterile	Other fungi
191	20	13	7	0
192	20	13	7	0
193	10	6	4	0
186	20	7	13	0
303	5	3	1	1 *Fusarium* sp.

A number of isolations from common and hairy vetch and field peas were made in the spring of 1948, 1949, and 1950, including "straight", curved, and falcate conidial types of *Colletotrichum* and *Gloeosporium* isolates. Those retained for study are listed below:

Isolate No.	Conidial shape	Source	Isolated by
186	"straight"	hairy vetch-L.S.U. Horticulture farm	Dr. J. G. Atkins
191	"	common vetch-L.S.U. Horticulture farm	" " " "
192	"	common vetch-L.S.U. Horticulture farm	" " " "
193	"	common vetch-L.S.U. Horticulture farm	" " " "
192-W	"	wild vetch-L.S.U. Horticulture farm	N. L. Horn
192-Y	"	yellow mutant of isolate 192	" " "
703	"	common vetch-Winnsboro, La.	" " "
704	"	common vetch-St. Joseph, La.	" " "

Isolate No.	Conidial shape	Source	Isolated by
303	curved	narrow-leaf vetch-Baton Rouge, La.	Dr. J. G. Atkins
498	"	vetch Baton Rouge, La.	" " " "
800	"	hairy vetch-L.S.U. Horticulture farm	N. L. Horn
801	"	hairy vetch-L.S.U. Horticulture farm	" " "
802	"	hairy vetch-L.S.U. Horticulture farm	" " "
803	"	hairy vetch-L.S.U. Horticulture farm	" " "
850	falcate	hairy vetch-St. Joseph, La.	" " "
212	"	Dixie Wonder pea-Baton Rouge, La.	Dr. J. G. Atkins
213	"	Dixie Wonder pea-Baton Rouge, La.	" " " "
851	"straight"	Singletary pea-L.S.U. Horticulture farm	N. L. Horn
852	"	hairy vetch-L.S.U. Horticulture farm	" " "
701	"	common vetch-St. Joseph, La.	" " "
702	"	common vetch-Winnsboro, La.	" " "
900	"	common vetch-Baton Rouge, La.	" " "
901	"	hairy vetch-Baton Rouge, La.	" " "

Isolates 186, 191, 192, 192-W, 192-Y, 703, and 704 were Colletotrichums with "straight" conidia; isolates 303, 498, 800, 801, 802, and 803 were C. villosum, the curved conidial type; isolates 212 and 213 were C. pisi and isolate 850 a Colletotrichum sp., all of the falcate type; isolates

851, 852, 701, 702, 900, and 901 were Gloeosporium sp. These cultures were maintained on potato-dextrose agar slants in the refrigerator until they were needed for inoculation tests.

INOCULATIONS AND PATHOGENICITY TESTS

Common and hairy vetch plants four weeks old were inoculated in the greenhouse with conidial suspensions of the isolates listed in the previous section to determine their pathogenicity. Conidial suspensions were prepared from potato-dextrose agar slants on which the isolates had been growing for ten days. After inoculation the plants were placed in a moisture chamber. Spots appeared on some of the plants within four days after inoculation. Seven days after inoculation the plants were examined and the severity of infection recorded. The data are presented in Table 2.

From the results of these inoculations it appeared that common vetch was very susceptible to the "straight" spored Colletotrichum sp.; in fact, many plants were killed within seven days after inoculation (Plate IV). Austrian Winter peas and Dixie Wonder peas were also susceptible to the same isolates, but not as susceptible as common vetch. Hairy vetch, on the other hand, showed some resistance to these "straight" spored isolates. The fungi produced spots on the foliage and stems but were not nearly as numerous and in no instance were any hairy vetch plants killed within seven days. However, hairy vetch was very susceptible to the isolates of C. villosum, while common vetch was resistant. Austrian Winter and Dixie Wonder peas were very resistant to C. villosum. No spots were produced on these plants within a week after inoculation, but a few were observed on senescent leaves two weeks after inoculation. Isolates 212 and 213 were slightly pathogenic to common and hairy vetch. The infection appeared as minute blackened spots less than one half mm.

Table 2.--The severity of infection caused by anthracnose isolates on vetch and field peas.

Isolate No.	Degree of infection on			
	Common vetch	Hairy vetch	Austrian Winter peas	Dixie Wonder peas
192	very severe	moderate	severe	severe
192-W	"	"	"	"
192-Y	"	"	"	"
703	"	"	"	"
704	"	"	"	"
303	mild	severe	none	none
498	"	"	"	"
800	"	"	"	"
801	"	"	"	"
802	"	"	"	"
803	"	"	"	"
850	none	none	"	"
212	slight	slight	"	"
213	"	"	"	"
851	none	none	"	"
852	"	"	"	"
701	"	"	"	"
702	"	"	"	"
900	"	"	"	"
901	"	"	"	"

in diameter, occurring on stems and petioles only. Few spots appeared on pea plants inoculated with isolates 212 and 213 and these were on senescent leaves. The spots were circular and up to several mm. in diameter and light brown in color. Isolate 850 produced no noticeable infection on any of the plants. From these observations it was concluded that the "straight" and curved conidial types of Colletotrichum were of major importance and that the falcate conidial types and the Gloeosporium sp. were weakly pathogenic or became established only on diseased or weak plants.

Isolates of the two types were used to artifically infest seed in order to determine the ability to produce foot rots. Seeds of common and hairy vetch were surface disinfested with clorox (one part clorox to four parts distilled water) for five minutes and dried. Individual lots of seeds were dipped in a conidial suspension of isolates 192 and 303, respectively, and planted in steamed soil in three-inch pots (ten seeds per pot). Other seed lots were planted in steamed soil with no inoculation as controls. The experiment was conducted in the greenhouse. One month after planting, the plants were removed from the soil and the basal portions of the stems were examined for foot rot. The results are given in Table 3.

The results in Table 3 showed that isolate 303 caused a foot rot of hairy vetch, but not common vetch. They were in agreement with previous inoculations; that is, hairy vetch was susceptible to isolate 303 when the fungus was applied as a conidial suspension to the foliage of the plants, whereas common vetch plants were resistant when inoculated by the same method. It was expected that isolate 192 would have been more effective in causing a foot rot of common vetch. But only ten per cent

of these plants showed symptoms. The low per cent of foot rot caused by isolate 192 on hairy vetch was in accordance with foliage inoculations of isolate 192 on hairy vetch. There were several possible

Table 3.--The occurrence of foot rot on common and hairy vetch plants inoculated with isolates 192 and 303, respectively.

Isolate No.	Common vetch		Hairy vetch	
	No. of plants observed	No. of plants with foot rot	No. of plants observed	No. of plants with foot rot
192	20	2	30	1
303	22	0	29	28
check	22	1	26	0

explanations regarding the one infected plant in the controls. Either there was contamination due to the splashing of conidia from adjacent pots containing infected plants, or it resulted from seed borne infection. No attempts were made, however, to determine the cause. Since the per cent infection on common vetch plants caused by isolate 192 was so much lower than expected, a similar experiment was conducted, using isolate 192 alone and introducing another treatment in which unsterilized seeds were planted in steamed soil. The results are given in Table 4. These data agreed with foliar inoculations showing that common vetch was very susceptible to isolate 192, whereas hairy vetch showed resistance to this isolate. The control plants remained healthy, perhaps because greater distances were maintained between control plants and inoculated plants.

A third method of inoculation was used to test the pathogenic effect of the "straight" and curved conidial type Colletotrichum spp. on common and hairy vetch. Oat seeds in 250 mm. flasks to which a

Table 4.--The occurrence of foot rot on common and hairy vetch caused by isolate 192.

Isolate No.	Common vetch		Hairy vetch	
	No. of plants observed	No. of plants with foot rot	No. of plants observed	No. of plants with foot rot
192	28	23	30	5
check[a]	25	0	27	0
check[b]	24	0	26	0

[a]Surface sterilized seed.

[b]Non-surface sterilized seed.

small amount of water was added, were autoclaved for one hour at 15 pounds pressure on two successive days. Into half the flasks were poured conidial suspensions of isolate 192 and into the other half were poured conidial suspensions of isolate 303. All were placed at 22°C for a month in order to allow the fungi to grow throughout the oats and thus produce a good source of inoculum. The inoculum of each isolate, respectively, was mixed with steamed soil in three-inch pots. Then common and hairy vetch seeds were surface disinfested with clorox and planted in the artifically infested soil. The treatments consisted of common vetch seeds planted in soil infested with isolate 192, common vetch seeds planted in soil infested with isolate 303, common vetch seeds planted in non-infested soil (controls), and hairy vetch seeds planted in soils similarly treated. Ten seeds were planted per pot with five replications. Seventeen days after the beginning of the experiment the plants were dug, washed, and examined for infection. Lesions occurred on the basal portion of the stems just above the cotyledons of a large number of the plants grown in infested soil. The results are given in Table 5.

Table 5.--The occurrence of basal stem spots on common and hairy vetch planted in soil infested with isolates 192 and 303, respectively.

Isolate No.	Pot No.	Common vetch		Hairy vetch	
		No. plants observed	No. plants infected	No. plants observed	No. plants infected
192	1	7	6	9	5
	2	10	10	8	7
	3	9	9	8	5
	4	7	6	10	7
	5	10	10	8	5
	Total	43	41	43	34
303	1	9	7	6	4
	2	9	4	9	6
	3	10	7	8	8
	4	9	7	9	8
	5	10	8	6	5
	Total	47	33	38	31
Check	1	10	0	6	0
	2	8	0	10	0
	3	10	0	8	0
	4	8	0	6	0
	5	9	0	8	0
	Total	45	0	38	0

The most severe infection occurred on common vetch stems when grown in soil infested with isolate 192. Some of these plants were rotted at the base and died before final data were obtained. Most of the other plants in this treatment contained numerous spots on the stems from the cotyledon upward to a distance of about two cm. These spots were tan surrounded by a reddish colored border, elliptical, sunken, up to five mm. long, and up to two mm. wide. Hairy vetch plants showed similar spots which were much smaller in size and number. None of the hairy vetch plants were killed.

Isolate 303 caused infection on both common and hairy vetch stems. The spots were numerous, small, reddish-brown streaks no longer than 1.5 mm. or wider than 0.5 mm. Often they coalesced to form a reddish-brown blotch. The hairy vetch stems were usually discolored as compared with the normal white portion of the controls. Common vetch plants were seldom discolored. No spots were observed on any of the control plants. Plate VI is a photograph of a few diseased and healthy plants used in this experiment.

Relationship of plant response and nodule formation to infection:

Since plants used in inoculation tests were grown in steamed soil, it was desirable to determine whether or not plants which were inoculated with nodule-forming bacteria reacted the same in resistance or susceptibility as plants not inoculated with nodule-forming bacteria. Therefore, the following test was carried out in the greenhouse with common and hairy vetch. Seeds were surface sterilized with diluted clorox for five minutes, then washed in distilled water. This was accomplished by placing the seeds in a small wire basket with sufficiently fine mesh so that the seeds did not fall through, and then

lowering the basket into the clorox deeply enough to completely cover the seeds. After five minutes the basket was removed from the solution, the seeds allowed to drain to remove the excess clorox, and the basket dipped into distilled water for several minutes with slight agitation to thoroughly wash the seeds. A portion of these seeds were inoculated with a commercial preparation of nodule-forming bacteria called "Nitrogin". The method used to inoculate with bacteria was similar to the one used to surface sterilize the seeds. Seeds were placed in a wire basket and dipped into a water suspension of the commercial preparation. Individual lots of inoculated seeds were planted in steamed and non-steamed soil in three-inch pots in the greenhouse. Surface sterilized seeds not inoculated with bacteria were planted in steamed and non-steamed soil in three-inchpots, respectively. Plantings were made at the rate of ten seeds per pot with four pots in each treatment.

Twenty-four days after planting half of the plants (in two pots) of each treatment was inoculated with a spore suspension of isolate 192 and the other half with isolate 303. All were placed in a moisture chamber for three days. Four days later the plants were examined carefully for possible differences in susceptibility among the different treatments. That is, hairy vetch plants whose seeds were inoculated with bacteria and inoculated with isolate 192 were compared with hairy vetch plants not previously inoculated with bacteria but inoculated with isolate 192. Common vetch plants from seeds inoculated with bacteria and inoculated with isolate 192 were compared with plants from seeds not inoculated with bacteria but inoculated with isolate 192. The same comparisons were made with plants inoculated with isolate 303.

All common vetch plants inoculated with isolate 192 regardless of treatment, responded similarly. Likewise, all hairy vetch plants inoculated with isolate 192 responded alike. Common vetch plants were severely injured or dead, but hairy vetch plants, although they contained numerous spots, were not severely injured. All common vetch and hairy vetch plants, respectively, inoculated with isolate 303 responded the same whether or not the seeds were treated with "Nitrogin". The hairy vetch plants were severely infected with leaf and stem spots while the common vetch plants contained fewer spots which were much smaller.

The roots of plants of each treatment were examined for nodules. All the plants whose seeds had been inoculated with "Nitrogin" had numerous nodules on the roots. Occasionally, several nodules were found on plants whose seeds had not been inoculated with "Nitrogin", but the majority of plants had no nodule formation on their roots.

Relationship of added nitrogen to the soil to infection:

Another test was conducted in the greenhouse to find out whether or not the addition of nitrogen to the soil in which common and hairy vetch plants were growing, would have any noticeable effect on the susceptibility or resistance of these plants. Seeds of common and hairy vetch, respectively, were surface sterilized with clorox and washed with distilled water and planted in steamed soil in three-inch pots (ten seeds per pot) on November 11, 1950. Potassium nitrate at the rates of one gram, two grams, and four grams in 1,000 ml. of distilled water, respectively, were added to each of four pots of common vetch and each of four pots of hairy vetch in 50 ml. amounts on the following dates: November 24 and 30, and December 4, 8, 10, and 11.

Between these times of the addition of nitrate solution a minimum amount of water was added to prevent the plants from wilting. Controls consisted of plants treated similarly, but 50 ml. of distilled water was added to the soil on the dates of the application of nitrate. On December 12, half of the plants, including two pots for each treatment, were inoculated with conidial suspensions of isolate 192, and the other half with isolate 303. These plants were placed in a moisture chamber in the greenhouse for 72 hours, then removed to a greenhouse bench and examined to determine any differences in susceptibility of the plants among the various treatments. Similar results were obtained as in the previous test. No noticeable differences were observed in resistance or susceptibility. All hairy vetch plants inoculated with isolate 192 appeared to react alike. All hairy vetch plants inoculated with isolate 303 showed a similar degree of infection. All common vetch plants inoculated with isolate 192 died. All common vetch plants inoculated with isolate 303 appeared to be equally infected.

MORPHOLOGICAL STUDIES

Isolate 192 was a representative culture of a number of "straight" conidial type Colletotrichum isolates which were collected from diseased vetch and field peas at several localities in Louisiana. This culture grew well on potato-dextrose agar at room temperatures nearly covering the agar in a nine cm. petri-dish in ten days. At first new transfers produced a sparse, white mycelial growth, but after several days the center of the colonies became black and much supressed. The diameter of the blackened areas increased with increased diameter growth of the colonies. These jet black formations resembled closely fitted sclerotial masses and were firm and crust-like. In old cultures the sclerotial-like masses were formed deep in the medium. The mycelium at the periphery of the colonies always appeared white so that older colonies consisted of a black center which became wrinkled with the age of the culture, surrounded by a band of white supressed mycelium. Setae were often present within the sclerotial masses. Most of the mycelial growth of these colonies was confined to the surface of the agar. Cultures of this type produced very few conidia and in the preliminary inoculation sufficiently concentrated conidial suspensions were hard to obtain. The isolate was grown on a variety of agar media, bean pods, and vetch stems, but on each medium few conidia were produced. Occasionally, spore masses were formed, but these were rare and usually only one to several per slant. Fortunately, however, a bright yellow, pigmented sector appeared in one of these cultures which produced conidia in abundance. Conidial masses of this new form were never observed; the conidia were produced on hyphal tips. Transfers

were made from this sector and the new isolate was designated as 192-Y. The growth of this isolate was similar to the original in some respects. Colonies grown on potato-dextrose agar produced mycelium which first appeared white like the 192 culture, was supressed and grew just as rapidly. Later, instead of black sclerotial-like masses being formed, the isolate produced a yellow pigmentation which increased in diameter with the increase in diameter growth of the cultures. These cultures were not crust-like, but were soft and easily pierced with a blunt instrument. Setae were never observed in colonies grown on potato-dextrose agar. A photograph of isolate 192 and 192-Y is shown on Plate IX.

Isolate 303 is a representative culture of a number of curved conidial isolates identified as Colletotrichum villosum. The original culture grew slowly on all media tested, never reaching a diameter greater than 20 mm. in ten days on any of the media. After transfer to fresh plates or slants the mycelium appeared white and supressed, spreading out flat over a small surface. Within a few days the center of the growth had produced black, pigmented mycelium, it too being much supressed. The black areas increased in diameter with increase in growth of the culture. Within seven days the blackened masses in the center of the culture became raised to form a large sclerotial clump rather than remain flattened. In older colonies the individual sclerotial masses were deep-seated in the agar. At the same time large numbers of conidial masses were produced in the center of the cultures. They varied from Ochraceous-buff of Ridgway to gray in color. Often the spore masses completely covered the centers of the cultures. The mycelium bordering the cultures remained white and supressed. The

cultures showed no reverse color. A photograph of this isolate is presented on Plate II.

Mycelium:

Young hyphae of isolate 192 were irregularly septate, hyaline and ranged from 0.5 u to 1.0 u in width. The older mycelium formed numerous chain-like cells, either barrel-shaped or spherical, thicker walled and some were as much as 5 u in diameter. These latter type cells contained a few yellowish-brown oil globules ranging up to several microns in diameter. The cells of the sclerotial masses were thick walled, uniformally dark brown to nearly black in color, and joined adjacent cells on more than one side, almost resembling the cells of a perithecium or a similar fruiting structure.

The mycelium of isolate 192-Y appeared to be nearly the same as isolate 192. Young hyphae were 0.5 u to 1.0 u in diameter, hyaline, and contained frequent septa. The cells of the oldest mycelium measured up to 4 u in diameter; the cells were swollen, mostly oval; a few appeared spherical, thick walled, and contained large yellow, pigmented bodies. These cultures contained no sclerotial masses and no dark brown colored cells.

The young hyphae of isolate 303 were hyaline, irregularly septate and measured up to 1.0 mm. in diameter. The oldest mycelium consisted mostly of thicker, oval to spherical chains of cells filled with large (up to 1.5 u) dark yellowish-brown pigmented bodies. The cells of the sclerotial masses consisted of chains of light to dark-brown cells uniformly colored, with few to no oil globules.

Conidia:

Measurements of conidia were made of isolate 192 from acervuli on inoculated common vetch plants grown in the greenhouse. The

conidial samples were obtained 14 days after the plants had been inoculated. Ten conidia only were measured from any one water mount, until the length and width of 50 conidia were recorded. These measurements are listed below:

	Length in u	Width in u		Length in u	Width in u
1	22.2	3.7	23	22.2	3.7
2	18.5	5.6	24	22.2	3.7
3	22.2	3.7	25	22.2	3.7
4	22.2	3.7	26	16.7	3.7
5	20.4	4.1	27	22.2	3.7
6	20.4	3.7	28	22.2	3.7
7	22.2	3.7	29	22.2	3.7
8	22.2	3.7	30	22.2	3.7
9	22.2	3.7	31	18.5	3.7
10	18.5	3.7	32	22.2	3.7
11	22.2	3.7	33	18.5	3.7
12	22.2	3.7	34	20.4	3.7
13	20.4	3.7	35	18.5	4.1
14	22.2	3.7	36	22.2	3.7
15	22.2	3.7	37	22.2	3.7
16	20.4	4.1	38	18.5	3.7
17	22.2	3.7	39	22.2	3.7
18	22.2	3.7	40	22.2	3.7
19	20.4	3.7	41	18.5	4.1
20	18.5	3.7	42	22.2	3.7
21	22.2	3.7	43	20.4	3.7
22	22.2	3.7	44	18.5	3.7

	Length in u	Width in u		Length in u	Width in u
45	20.4	3.7	48	22.2	3.7
46	22.2	3.7	49	22.2	3.7
47	20.4	3.7	50	22.2	3.7
			Average	21.2	3.8
			Mostly	22.2	3.7

Conidia from cultures of isolate 192 growing on potato-dextrose agar were also measured and listed below:

	Length in u	Width in u		Length in u	Width in u
1	22.2	3.7	18	25.9	3.7
2	22.6	3.7	19	22.2	3.7
3	22.6	3.7	20	22.2	3.7
4	18.9	3.7	21	16.7	4.1
5	21.5	3.7	22	22.2	3.7
6	20.4	4.1	23	18.5	3.7
7	22.2	3.7	24	20.4	4.1
8	22.2	3.7	25	24.1	3.7
9	20.4	3.7	26	22.2	3.7
10	22.2	3.7	27	16.7	3.7
11	22.2	3.7	28	22.2	3.7
12	21.5	3.7	29	21.5	3.7
13	22.2	3.7	30	22.2	3.7
14	22.2	3.7	31	24.1	3.7
15	18.9	4.1	32	18.5	3.7
16	22.2	3.7	33	18.9	3.7
17	22.2	3.7	34	22.6	3.7

	Length in u	Width in u		Length in u	Width in u
35	22.2	3.7	43	21.5	3.7
36	22.2	3.7	44	22.2	3.7
37	17.8	3.7	45	20.4	3.7
38	18.5	4.1	46	22.2	3.7
39	20.4	3.7	47	22.2	3.7
40	22.2	3.7	48	18.5	3.7
41	22.2	3.7	49	22.2	3.7
42	22.2	3.7	50	20.4	3.7
			Average	21.3	3.7
			Mostly	22.2	3.7

Conidia of isolate 192-Y were measured, since this isolate produced many large conidia on potato-dextrose agar in comparison to those of isolate 192. These are listed below:

	Length in u	Width in u		Length in u	Width in u
1	25.9	5.6	13	20.4	3.7
2	22.2	4.4	14	18.5	3.7
3	22.2	4.4	15	35.2	3.7
4	14.8	3.7	16	22.2	4.4
5	29.6	5.6	17	22.2	4.4
6	22.2	4.4	18	22.2	3.7
7	20.4	3.7	19	22.2	3.7
8	31.5	5.6	20	18.5	3.7
9	37.0	5.6	21	20.4	3.7
10	33.3	5.6	22	27.8	3.7
11	18.5	3.7	23	18.5	3.7
12	22.2	4.4	24	22.2	4.4

	Length in u	Width in u		Length in u	Width in u
25	22.2	4.4	38	18.5	4.4
26	18.5	4.4	39	18.5	3.7
27	20.4	3.7	40	22.2	3.7
28	22.2	4.4	41	25.9	4.4
29	27.8	4.4	42	20.4	4.4
30	22.2	4.4	43	22.2	3.7
31	20.4	3.7	44	25.9	4.4
32	20.4	4.4	45	22.2	4.4
33	33.3	5.6	46	22.2	3.7
34	29.6	3.7	47	16.7	3.7
35	22.2	4.4	48	22.2	3.7
36	22.2	4.4	49	22.2	3.7
37	22.2	4.4	50	22.2	3.7
			Average	23.1	4.2
			Maximum	37.0	5.6
			Minimum	14.8	3.7

Isolate 303 was grown on potato-dextrose agar and conidia examined when they were produced freely. The measurements are recorded below:

	Length in u	Width in u		Length in u	Width in u
1	22.2	3.7	8	20.4	3.7
2	18.5	4.4	9	18.5	4.4
3	22.2	3.7	10	22.2	4.4
4	18.5	3.7	11	16.7	4.4
5	20.4	3.7	12	20.4	4.4
6	24.1	4.4	13	22.2	3.7
7	18.5	5.6	14	18.5	4.4

	Length in u	Width in u		Length in u	Width in u
15	18.5	4.4	33	18.5	4.4
16	18.5	4.4	34	22.2	4.4
17	20.4	3.7	35	16.7	4.4
18	20.4	3.7	36	16.7	4.4
19	20.4	4.1	37	20.4	3.7
20	22.2	4.4	38	18.5	4.4
21	20.4	3.7	39	16.7	3.7
22	22.2	4.4	40	22.2	4.4
23	14.8	4.1	41	22.2	5.6
24	18.5	4.4	42	18.5	4.4
25	22.2	4.4	43	14.8	3.7
26	22.2	3.7	44	18.5	3.7
27	18.5	3.7	45	18.5	3.7
28	16.7	3.7	46	22.2	4.4
29	18.5	3.7	47	18.5	3.7
30	18.5	3.7	48	18.5	4.4
31	22.2	5.6	49	20.4	5.6
32	18.5	4.1	50	18.5	4.4
			Average	19.5	4.2
			Mostly	18.5	4.4

The conidia of isolate 192 were hyaline, unicellular, "straight", and bluntly tapered at the tips. Conidia produced on potato-dextrose agar ranted from 16.7 u to 25.9 u in length, 3.7 to 4.1 u in width, averaged 21 u x 3.7 u, and the most common size was 22.2 u x 3.7 u.

Conidia produced by the fungus on the plant ranged from 16.7 u to 22.2 u in length to 3.7 u to 5.6 u in width, averaged 21.2 u x 3.8 u, and the most common size was 22.2 x 3.7 u. Conidial masses were rare when the fungus was grown on agar, but when present were Ochraceous-buff of Ridgway. Conidial masses from plant spots were, also, of similar color.

The conidia of isolate 303 were unicellular, hyaline, curved, and less bluntly tapered at the tips than 192. Of the conidia measured the range was 14.8 u to 24.1 u in length and 3.7 u to 5.6 u in width, the average size being 18.5 u x 4.2 u. The most common size was 18.5 u x 4.4 u.

The two conidial types are represented in Plate IX.

Setae:

Infected sections of common vetch stems which were taken from plants inoculated in the greenhouse with isolate 192 were placed in moisture chambers in the laboratory. Within five to six days numerous setae developed from the spots. These setae were dark brown at the bulbous base, becoming lighter in color toward the tips. They were bluntly tapered to a point and contained from one to four septa. The measurements of 28 setae from infected plant material are listed below (the diameter measurements are made across the bulbous base):

	Length in u	Width in u	No. septa		Length in u	Width in u	No. septa
1	151.7	8.4	3	5	136.9	6.5	3
2	162.8	6.5	3	6	70.3	7.4	2
3	157.3	8.4	3	7	125.8	8.4	2
4	199.8	8.4	4	8	136.9	6.5	2

	Length in u	Width in u	No. septa		Length in u	Width in u	No. septa
9	112.9	6.5	2	19	101.8	6.5	2
10	125.8	6.5	3	20	81.4	6.5	1
11	166.5	7.4	3	21	164.7	7.4	3
12	138.8	7.4	4	22	162.8	6.5	4
13	149.9	6.5	3	23	164.7	6.5	4
14	111.0	6.5	3	24	88.8	6.5	2
15	129.5	8.4	3	25	107.3	6.5	2
16	94.4	6.5	1	26	68.5	6.5	2
17	61.6	6.5	2	27	111.1	6.5	2
18	98.1	6.5	2	28	128.2	7.4	3

Measurements of setae from isolate 192 grown on potato-dextrose agar were also made.

	Length in u	Width in u	No. septa		Length in u	Width in u	No. septa
1	133.2	6.5	3	11	148.0	7.4	3
2	107.3	6.5	3	12	166.5	7.4	4
3	85.1	7.4	2	13	118.4	9.3	4
4	167.3	6.5	5	14	155.4	7.4	3
5	74.0	6.5	2	15	144.3	7.4	5
6	92.5	6.5	2	16	155.4	7.4	4
7	155.4	6.5	4	17	163.9	8.4	5
8	107.3	7.4	2	18	92.5	6.5	1
9	122.1	6.5	3	19	133.2	7.4	4
10	111.0	9.3	3	20	148.0	7.4	3

The setae obtained from cultures grown on agar contained up to five septa. Although no record of setae with five septa was recorded here such setae had been observed previously to occur on infected host

tissue. From the measurements recorded here the setae ranged from 61.6 u to 199.8 u long and 6.5 u to 9.3 u wide. They averaged 126.9 u x 7.1 u.

Isolate 192-Y had never been observed to produce setae either in culture or on host tissue.

The setae of isolate 303 were similar in shape and color as those described for isolate 192. On the other hand, the setae of isolate 303 were somewhat shorter on the average and usually contained fewer septa. Measurements were made of setae obtained from cultures grown on potato-dextrose agar and from infected tissue of hairy vetch plants inoculated in the greenhouse. These measurements were as follows:

	From potato-dextrose agar			From host tissue		
	Length in u	Width in u	No. septa	Length in u	Width in u	No. septa
1	144.3	7.4	3	125.8	7.4	2
2	66.6	6.5	3	129.5	7.4	2
3	77.7	7.4	3	129.5	7.4	3
4	144.3	9.3	3	129.5	7.4	2
5	96.2	7.4	1	106.3	6.5	2
6	55.5	7.4	1	136.9	8.1	3
7	92.5	7.4	2	81.4	6.5	1
8	111.0	7.4	2	103.6	6.5	1
9	111.0	7.4	1	118.4	7.4	1
10	118.4	6.5	2	107.3	7.4	2
11	122.1	7.4	2	133.2	8.1	2
12	81.4	7.4	2	133.2	7.4	3
13	85.1	7.4	2	125.8	6.5	4
14	148.0	7.4	3	140.6	8.1	4
15	118.4	7.4	1	129.5	7.4	3

	From potato-dextrose agar			From host tissue		
	Length in u	Width in u	No. septa	Length in u	Width in u	No. septa
16	107.3	7.4	2	92.5	6.5	1
17	118.4	7.4	3	125.8	8.1	3
18	92.5	7.4	2	133.2	9.3	2
19	103.6	7.4	2	96.2	6.5	1
20	70.3	6.5	1	99.9	6.5	2
21	55.5	6.5	1	148.0	7.4	3
22	99.9	7.4	2	144.3	7.4	3
23	51.8	6.5	1	103.6	6.5	4
24	53.7	7.4	1	133.2	6.5	3
25	74.0	6.5	2	136.9	7.4	2
26	111.0	7.4	3	99.9	6.5	2
27	88.8	7.4	2	114.7	6.5	2
28	92.5	7.4	2	66.6	6.5	1
29	77.7	7.4	1	74.0	6.5	1
30	108.6	7.4	3	122.1	7.4	3
31	129.5	7.4	2	107.3	7.4	1
32	81.4	7.4	1	99.9	8.1	1
33	136.9	7.4	4	103.6	7.4	2
34	66.6	8.1	2	85.1	6.5	1
35	77.7	7.4	2	111.0	6.5	1
36	111.0	7.4	2	74.0	6.5	1
37	103.6	7.4	2	114.7	7.4	2
38	85.1	6.5	1	177.6	8.1	3
39	96.2	7.4	2	111.0	7.4	1
40	122.2	7.4	2	162.8	9.3	3
41	107.3	7.4	2	140.6	7.4	3
42	144.3	9.3	3	155.4	8.1	3

	From potato-dextrose agar			From host tissue		
	Length in u	Width in u	No. septa	Length in u	Width in u	No. septa
43	118.4	6.5	2	140.6	7.4	3
44	103.6	6.5	2	96.2	6.5	1
45	74.0	6.5	1	166.5	8.1	4
46	92.5	7.4	1	99.9	6.5	1
47	129.5	7.4	3	118.4	7.4	2
48	114.7	6.5	2	129.5	8.1	2
49	133.2	7.4	2	148.0	8.1	3
50	140.6	8.1	3	107.3	7.4	1

From the combined measurements the average was 109.8 u x 7.3 u. They ranged from 55.5 u to 177.6 u long and 6.5 u to 9.3 u wide.

Acervuli:

Common vetch plants were inoculated with isolates 192 and 303, respectively, in the greenhouse. The plants were carefully handled during the inoculation and incubation processes so that contamination was avoided. Seven days later stem sections of each were brought into the laboratory, surface disinfested, placed in moist chambers, and exposed to blue light. As soon as numerous setae appeared on the stem tissue, stem sections were placed in formalin acetic acid killing solution. Paraffin sections were made from these stems. Measurements of the acervuli of both isolates are recorded below:

Isolate 303		Isolate 192	
170.2	81.4	74.0	111.0
74.0	114.7	81.4	55.5
133.2	125.8	88.8	88.8
85.1	99.9	85.1	92.5
133.2	122.1	74.0	81.4
88.8	103.6	74.0	51.8

Isolate 303		Isolate 192	
92.5	55.5	81.4	66.6
111.0	118.4	59.2	103.6
129.5	155.4	74.0	74.0
148.0	129.5	118.4	92.5
151.7	185.0	74.0	107.3
148.0	148.0	125.8	96.2
133.2	118.4	74.0	114.7
122.1	103.6	111.0	88.8
111.0	118.4	74.0	81.4
Average	120.4	Average	85.8

These acervuli were individually formed with one to many setae seated in the center. Both isolate 192 and 303 produced acervuli which were similar in structure, but those produced by the latter were generally somewhat larger in diameter (Plate X).

PHYSIOLOGICAL STUDIES

Temperature:

The growth rates of isolate 192 and 303 were compared at various temperatures. This was accomplished by two methods. Biscuit cuts of mycelium of each isolate, respectively, were placed on agar in petri-plates, which were maintained at controlled temperatures for seven days, and the cultures which developed measured. The other method consisted of determining the per cent germination of conidia which were spread over the surface of agar in petri-plates and maintained at controlled temperatures for seven days. The former will be discussed first.

Isolates 192 and 303, respectively, were grown on non-acid potato-dextrose agar for nine days. From the periphery of the cultures biscuit cuts of mycelium were made with a special instrument so that each piece of inoculum was of uniform size (3 mm. in diameter). Potato-dextrose agar in 9 cm. petri-plates was inoculated with biscuit cuts of the two isolates, respectively. Three inoculated plates of each isolate were placed in the following controlled incubators: 7°, 15°, 22°, 25°, 27°, 33°, and 36° C. Seven days later the plates were removed from the incubators and the diameter growth of the cultures measured in mm. (Table 6).

The data in Table 6 indicated that isolate 192 grew more rapidly than isolate 303 under the specified conditions. Cultures of the former produced a much greater growth in diameter at 15°, 22°, 25°, 27°, 33°, and 36° C than those of isolate 303; in fact, the ratio was greater than three to one at 25°, 27°, and 33° C, and nearly three to

Table 6.--The diameter growth in mm. of isolates 192 and 303, respectively, on potato-dextrose agar at various temperatures.

Isolate No.	Petri-plate No.	Temperature, °C 7	15	22	25	27	33	36
192	1	3.0[a]	20.0	36.0	37.0	41.0	18.0	15.0
	2	3.0[a]	17.0	35.0	40.0	42.0	18.0	20.0
	3	3.0[a]	20.0	37.0	36.0	35.0	19.0	18.0
	Average	3.0[a]	19.0	36.0	38.7	39.3	18.3	17.7
303	1	3.0[a]	7.0	14.0	10.0	10.0	3.0[a]	3.0[a]
	2	3.0[a]	6.0	12.0	13.0	8.0	3.0[a]	3.0[a]
	3	3.0[a]	7.0	12.0	9.0	7.0	3.0[a]	3.0[a]
	Average	3.0[a]	6.6	12.7	10.7	8.3	3.0[a]	3.0[a]

[a]These measurements were the diameters of the inoculum discs and actually represented no measureable diameter growth.

one at 15° and 22° C. It should be pointed out here, as will be shown later, that potato-dextrose agar was as favorable as any medium tested for the growth of these two isolates. Since isolate 303 is a much slower growing isolate, the use of potato-dextrose agar without some remark of this nature might have suggested that the difference in growth of the two isolates was due to the type of medium used. In this experiment the minimum growth for both isolates was 15° C. No growth of either isolate was measureable at 7°C. However, isolate 192 produced considerable growth at 33° and 36°C, whereas no growth was detected at these temperatures in the case of isolate 303. The optimum growth for each isolate ranged between 22° and 27°C.

This experiment was repeated except that the number of replications was increased to five and an additional treatment was used (18°C). The same procedure was followed, including the preparation of the agar medium. The results of this test are given in Table 7.

Table 7.--The diameter growth in mm. of isolates 192 and 303, respectively, on potato-dextrose agar at various temperatures.

Isolate No.	Petri-plate No.	Temperature, °C 7	15	18	22	25	27	33	36
192	1	3.0[a]	19.0	26.0	36.0	41.0	38.0	18.0	14.0
	2	3.0[a]	20.0	25.0	38.0	38.0	37.0	21.0	13.0
	3	3.0[a]	25.0	27.0	39.0	42.0	42.0	22.0	17.0
	4	3.0[a]	24.0	23.0	36.0	39.0	37.0	22.0	18.0
	5	3.0[a]	22.0	24.0	38.0	41.0	37.0	23.0	16.0
	Average	3.0[a]	22.0	25.0	37.5	40.2	38.2	21.2	15.6
303	1	3.0[a]	5.0	11.0	13.0	15.0	15.0	3.0[a]	3.0[a]
	2	3.0[a]	7.0	9.0	9.0	14.0	13.0	3.0[a]	3.0[a]
	3	3.0[a]	7.0	10.0	15.0	10.0	9.0	3.0[a]	3.0[a]
	4	3.0[a]	9.0	8.0	9.0	11.0	11.0	3.0[a]	3.0[a]
	5	3.0[a]	7.0	12.0	10.0	12.0	10.0	3.0[a]	3.0[a]
	Average	3.0[a]	7.0	10.0	11.2	12.4	11.6	3.0[a]	3.0[a]

[a]These measurements were the diameters of the inoculum discs and actually represented no measureable diameter growth.

The data in Table 7 indicated that the growth of the two isolates varied considerably. The optimum temperatures for growth were approximately the same for both isolates. But no measureable growth of the

latter occurred at or above 33°C, while the growth of the former at 33°C and 36°C was fairly good as compared to the growth at optimum temperatures. Cultures of isolate 192 grew three times the diameter or greater than cultures of isolate 303 at 15°, 22°, 25°, and 27°C. Outstanding is the fact that cultures of isolate 192 grew at temperatures above the maximum growth of isolate 303.

To show further the difference between isolates 192 and 303 in relation to the effect of temperature on their growth, conidia of each, respectively, were dispersed over potato-dextrose agar in petri-dishes and placed in controlled temperature chambers of 7°, 15°, 18°, 22°, 25°, 27°, 33°, and 36°C. Forty-eight hours later the plates were examined in order to determine the percentage germination. The per cent germination was determined by examining 100 conidia of both isolates from each treatment. The results are given in Table 8.

Table 8.--Per cent germination of conidia of isolates 192 and 303 at various temperatures.

Isolate No.	Temperature, °C							
	7	15	18	22	25	27	33	36
192	0	0	5	89	93	96	84	67
303	21	52	91	92	95	94	21	0

The results (Table 8) indicated that the optimum temperatures for conidial germination of both isolates were about the same, that is, between 22° and 27°C. The temperatures required for optimum growth in this test corresponded with the temperatures necessary for optimum development of cultures started with biscuit cuts of mycelium as inoculum.

Conidia of isolate 192 germinated readily at 33°C, whereas, germination of isolate 303 decreased abruptly at the same temperature. Germination of isolate 192 was reduced greatly at temperatures below 22°C, and similar conidia failed to germinate at 15° and 7°C. On the other hand the conidia of isolate 303 germinated well at 18°C and as much as 22 per cent at 7°C.

The experiment was repeated except that two additional treatments (30° and 40°C) were included. Similar results were obtained. The conidia were dispersed on potato-dextrose agar, allowed to remain in the controlled temperature chambers for 48 hours, and then counts were made in the same manner previously stated. The data was recorded in Table 9.

Table 9.--Per cent germination of conidia of isolate 192 and 303 at various temperatures.

Isolate No.	Temperatures, °C									
	7	15	18	22	25	27	30	33	36	40
192	0	0	13	79	90	91	92	80	40	6
303	22	64	87	93	94	90	92	63	0	0

From the data contained in Tables 8 and 9 it is evident that of the two isolates the conidia of isolate 192 germinated more readily at temperatures above the optimum than those of isolate 303. On the other hand germination of isolate 303 exceeded that of isolate 192 at temperatures below the optimum. The conidia of both isolates germinated well at 22°, 25°, 27°, and 30°C. The most significant feature was that conidia of isolate 192 germinated at temperatures above 33° while those of isolate 303 did not. Just as important was the fact that conidia of isolate 192 did not germinate at temperatures below 18°C whereas those of isolate 303 did.

Growth on agar media:

The growth of isolates 192 and 303 were compared on potato-dextrose, carrot, bean, white corn meal, yellow corn meal, oatmeal, and Czapek's agars. Common vetch and hairy vetch decoction agars were also used. The carrot, bean, white corn meal, yellow corn meal, and oatmeal agars were prepared similarly. Each contained 20 grams of agar and the extraction from 60 grams of bulk material made up to 1,000 ml. of water. The potato-dextrose agar consisted of 20 grams of agar, 20 grams of dextrose, and the extraction from 200 grams of potatoes made up to 1,000 ml. of water. Czapek's agar consisted of 20 grams of agar and the following amounts of materials made up to 1,000 ml. of water: $Mg\ SO_4 \cdot 7\ H_2O$, 0.5 grams; KH_2PO_4, 1.0 grams; KCl, 0.5 grams; $NaNO_3$, 0.01 grams; $FeSO_4$, 0.01 grams; and dextrose, 0.3 grams. The vetch decoction agars were prepared as follows: 200 grams of freshly harvested stems and leaves were ground to pulp in a Waring Blendor with 500 ml. of water. The roughage was filtered off and the filtrate made up to 1,000 ml. of solution to which 15 grams of agar was added. In all cases the media were autoclaved at 15 pounds pressure for 20 minutes.

The usual procedure for inoculation was followed by placing biscuit cuts of mycelium of isolate 192 and 303, respectively, on the various agars in petri-dishes. All cultures were maintained at 25°C for 7 days at which time measurements in diameter of the cultures were recorded. Five replications were used and the results are tabulated in Table 10.

Table 10.—Diameter growth in mm. of isolates 192 and 303 on various media at 25°C for 7 days.

Isolate No.	Petri plate No.	PDA	Carrot	Oatmeal	Yellow corn meal	Bean	Czapek's	White corn meal	Common vetch decoction	Hairy vetch decoction
192	1	22.0	24.0	33.0	25.0	33.0	22.0	42.0	28.0	30.0
	2	35.0	27.0	32.0	24.0	25.0	28.0	34.0	25.0	32.0
	3	55.0	25.0	30.0	25.0	32.0	25.0	35.0	29.0	29.0
	4	30.0	39.0	35.0	37.0	31.0	25.0	38.0	30.0	32.0
	5	45.0	30.0	31.0	33.0	29.0	29.0	39.0	30.0	29.0
	Average	34.7	29.0	32.2	28.8	30.0	25.8	37.6	28.4	30.4
303	1	14.0	16.0	13.0	15.0	16.0	4.0	16.0	11.0	16.0
	2	16.0	18.0	13.0	12.0	16.0	5.0	17.0	14.0	12.0
	3	18.0	19.0	12.0	12.0	15.0	4.0	15.0	13.0	15.0
	4	19.0	20.0	14.0	13.0	16.0	4.0	12.0	12.0	16.0
	5	13.0	19.0	15.0	14.0	14.0	4.0	15.0	11.0	18.0
	Average	16.0	18.4	13.4	13.2	15.4	4.2	15.0	12.4	15.4

From the data in Table 10 it is obvious that isolate 192 grew much faster than isolate 303. Isolate 192 grew best on potato-dextrose and white corn meal agars. Carrot and potato-dextrose agars gave the best growth of isolate 303. Both isolates grew poorly on Czapek's agar. The radial growth of isolate 303 was greatly reduced on Czapek's as compared with the other agars. Conidial production was about the same on all media.

Growth on liquid media:

The growth rates of isolates 192 and 303 were compared in Fries liquid medium supplemented with yeast extract. Neither isolate produced any noticeable growth in Fries medium alone or in Fries medium supplemented with casein in 10 days. Since both isolates failed to grow in the absence of yeast extract within this period of time it was assumed that both isolates required certain vitamins for growth. Fries medium alone was prepared with the following amounts of materials made up to 1 liter: NH_4 tartrate, 5 grams; NH_4NO_3, 1 gram; KH_2PO_4, 1 gram; $MgSO_4 \cdot 7\ H_2O$, 0.5 gram; NACl, 0.1 gram; $CaCl_2 \cdot 2\ H_2O$, 0.13 gram; trace elements B, 0.01 mg.; Cu 0.1 mg.; Mn, 0.02 mg.; Mo, 0.02; Zn, 2.0; dextrose 15 grams. When Fries solution was supplemented with yeast extract, 2.5 grams of the latter per liter of solution was used. Casein was added at the rate of 5 grams per liter.

Twenty ml. of Fries solution supplemented with yeast extract was poured into 125 ml. flasks (Erlenmeyer), plugged with cotton, and autoclaved at 15 pounds pressure for 20 minutes. When the medium was sufficiently cooled, conidia suspensions of isolates 192 and 303, respectively, were transferred to contents of these flasks. One loop

at a concentration of 50,000 conidia per ml. was transferred to 20 ml. of medium. Conidia were obtained from colonies which had been growing on potato-dextrose agar slants for seven days in the following manner. Several milliliters of 8 per cent sterile saline solution was poured into a tube over the agar. The surface of the culture was scraped slightly with a sterile needle to loosen the conidia and form a conidial suspension. This suspension was filtered through a cotton plug into a sterilized centrifuge tube to remove as much debris as possible. After suspensions of both isolates were obtained in this way, they were centrifuged for 20 minutes. The conidia formed a mass at the bottom of the tube; the liquid was decanted off, and the conidia resuspended in 8 per cent sterile saline solution by bubbling air through the added liquid with a sterile pipette. All glassware used was cleaned with sulfuric-acid and potassium dichromate cleaning solution and washed three times in distilled water. The pH after autoclaving was pH 5.5.

Loop transfers of the concentrated conidial suspensions were made to the flasks containing Fries solution. Four days later fungus mats of isolate 192 were removed from four flasks, washed thoroughly with distilled water, and oven dried at 90°C. This was repeated up to and including the seventh day and again on the ninth day. The dry weights of the mats were recorded (Table 11).

Isolate 192 grew fairly rapidly in the liquid medium; isolate 303 did not. Since the latter grew slowly the mycelial contents of three flasks were combined, oven dried, and weighed in order to get a large enough sample that could be handled conveniently. The first records of the weights of isolate 303 were made the fifth day after transfer and

not again until six days later. The mats weighed on the twelfth day were obtained from one flask for each sample. The final weights of mats were made on the fifteenth day. The results are recorded in Table 11.

Table 11.--Weights of mycelial mats of isolates 192 and 303 grown in Fries solution supplemented with yeast extract.

Isolate No.	No. days after transfer	Dry weight of samples in grams 1	2	3	4	average
192	4	.0095	.0127	.0152	.0087	.0115
	5	.0272	.0239	.0290	.0275	.0269
	6	.0395	.0643	.0594	.0511	.0536
	7	.0802	.0753	.0644	.0615	.0704
	9	.0268	.0407	.0619	.0224	.0370
303	5	.0061[a]	.0030[a]	.0029[a]	.0046[a]	.0011[a]
	12	.0107	.0070	.0084	.0086	.0087
	15	.0092	.0103	.0100	.0084	.0095

[a]A composite of mycelial mats from three flasks, the average, however, in terms of the weight of one flask.

Growth curves were made from the average dry weights taken from Table 11 (Plate VII). The growth rate of isolate 192 reached a maximum about the seventh day after transfer to the solutions, then decreased rapidly. Obviously, isolate 192 produced considerably more growth than isolate 303. Although the rates of growth for isolate 303 after nine days were not included on the graph the data in Table 11 indicated that the average growth of isolate 303 up to 15 days did not increase very much. Therefore, no more cultures of isolate 303 were

weighed. Consequently, the maximum growth rate of isolate 303 was not determined. From previous observations of the growth of isolate 303, it was assumed that the growth of this isolate would exceed little if any above that produced on the fifteenth day.

A similar experiment was conducted in the laboratory, but with the following alterations: casein was added to the medium at the rate of two grams per liter; the pH of the medium after autoclaving was pH 6.2; and the conidial concentration in suspension was 40,000 conidia per ml. The results are presented in Table 12.

Table 12.—Weights of mycelial mats of isolates 192 and 303 grown in Fries medium supplemented with yeast extract and casein.

Isolate No.	No. days after transfer	Dry weight of samples in grams 1	2	3	4	average
192	5	.0202	.0143	.0170	.0185	.0175
	6	.0188	.0198	.0266	.0262	.0228
	7	.0194	.0198	.0283	.0235	.0227
	8	.0300	.0305	.0338	.0340	.0321
	9	.0468	.0564	.0512	.0445	.0497
	10	.0530	.0440	.0644	.0597	.0552
	11	.0649	.0465	.0509	.0579	.0550
303	5	.0035	.0014	.0033	.0037	.0030
	6	.0030	.0041	.0032	.0028	.0035
	7	.0037	.0065	.0066	.0074	.0060
	8	.0084	.0071	.0141	.0075	.0092
	9	.0141	.0128	.0114	.0102	.0121
	10	.0151	.0179	.0168	.0135	.0158
	11	.0139	.0157	.0161	.0177	.0158

Although the growth rate of isolate 303 was increased in this medium and that of isolate 192 was somewhat reduced as compared to the growth on the medium used in the previous experiment, the growth of both isolates followed the same general pattern. The growth rate of isolate 192 far exceeded that of isolate 303.

Since Fries medium or Fries medium supplemented with casein was not suitable for the growth of isolates 192 or 303, several vitamins were added to solutions used in the above experiment that contained Fries solution supplemented with casein. Four vitamins alone and in all possible combinations were added to the solutions. These vitamins, biotin, nicotinamide, pyridoxine, and thiamine, were autoclaved for ten minutes at 15 pounds pressure and added at the rate of one fifth ml. of one part per 100 concentration per flask. The flasks were then incubated at 27°C. Solutions to which no vitamins were added were used as controls. Twenty-one days after the beginning of the experiment the solutions were examined. All solutions seeded with isolates 192 and 303, respectively, appeared to contain approximately the same amount of mycelial growth. No dry weights were made of the fungus mats since there was no obvious differences between treatments and controls. The assumption was made that both isolates required some vitamin or vitamins either independent of or in addition to those used here. Furthermore, both isolates will grow eventually in Fries solution or in Fries solution supplemented with casein, but only with difficulty. Growth was somewhat better in the latter.

Conidia were germinated in sterilized, distilled water and Fries medium supplemented with yeast extract and casein, respectively. The method used was as follows: one drop of the liquid was placed on a

glass slide to which was added several hundred conidia; a cover slip was placed over this suspension which was kept in a moist atmosphere at 22°C. The conidia were examined periodically to determine the time and method of germination. Conidia of isolate 192 germinated within 8 hours in the supplemented Fries medium and within 15 hours in the sterile distilled water. Conidia of isolate 303 germinated within 15 hours in the supplemented Fries medium and within 24 hours in the water. Drawings of germinating conidia are presented in Plate VIII.

Acid tolerance:

In preliminary studies results indicated that isolate 192 was relatively tolerant to acid media; that is, the fungus tolerated a concentration of three drops of 50 per cent lactic acid in 15 ml. of potato-dextrose agar, although growth was reduced on such a medium. Weimer (29) pointed out that Colletotrichum villosum did not withstand acid conditions as low as one drop of 50 per cent lactic acid in 15 ml. of agar.

Therefore, isolate 192 was grown at 22°C on potato-dextrose agar to which 1, 2, and 3 drops, respectively, of 50 per cent lactic acid was added to 15 ml. of medium after autoclaving. The agars were poured into 9 cm. petri-plates and inoculated with mycelium of isolate 192. The experiment was conducted so that each piece of inoculum was the same size. This was accomplished by the method described previously in which biscuit cuts of mycelium were used as inoculum. The discs were inverted so that the surface mycelium of the inoculum lay touching the agar in the petri-dishes. Measurements of the diameter growth of the colonies which developed were made seven days after inoculation (Table 13).

Table 13.--The effect of lactic acid (50 per cent), added at the rate of 0, 1, 2, and 3 drops per 15 ml. of medium, on the growth of isolate 192 at 22°C.

Petri-plate No.	Diameter of colonies in mm. No. of drops of acid			
	0	1	2	3
1	47.0	48.0	41.0	26.0
2	46.0	45.0	43.0	23.0
3	48.0	44.0	43.0	26.0
average	47.0	46.0	42.0	25.0

The fungus grew nearly as well on the medium containing one or two drops of acid as on the medium to which no acid was added. Media containing three drops of acid reduced the growth by one half. (Plate VII).

To confirm these results the same isolate was tested again under similar conditions except that the plates were held at 27°C and the number of replications increased to six. The results are given in Table 14.

The results of the second experiment did not agree exactly with the first test. The average growth on the medium with two drops of acid in the first test was over twice as much as in the second test. Also, the average growth on the medium with three drops of acid in the first test was over three times as much as in the second test. Since the acid and the medium were obtained from the same source, the only explanation of these results was the fact that the two experiments were conducted under different temperature conditions. However, the fungus

was shown previously to grow as well at 22°C as at 27°C. Regardless of these differences, the fact remains that the isolate did tolerate these acid conditions.

Table 14.--The effect of lactic acid (50 per cent), added at the rate of 0, 1, 2, and 3 drops per 15 ml. of medium, on the growth of isolate 192 at 27°C.

Petri-plate No.	Diameter of colonies in mm. No. of drops of acid			
	0	1	2	3
1	58.0	41.0	23.0	9.0
2	50.0	40.0	22.0	8.0
3	51.0	41.0	23.0	7.0
4	54.0	45.0	28.0	8.0
5	50.0	46.0	25.0	10.0
6	6.0	42.0	23.0	9.0
average	53.5	42.5	24.0	8.5

A comparison was made between the growth of isolates 192 and 303 on potato-dextrose agars which ranged from pH 4.8 to pH 7.6. Potato-dextrose agar was prepared and divided into seven portions to which were added sufficient amounts of acid, base, and buffer in order to obtain this pH range. The method used was outlined by Gortner (12). Included were pH 4.8, 5.2, 6.3, 7.0, 7.6, 8.2, and 8.6. The pH readings were made after autoclaving. The agar was poured into petri-plates and inoculated with biscuit cuts of mycelium three mm. in diameter taken from the periphery of cultures of isolates 192 and 303, respectively, and placed at 25°C. The inoculum was grown on non-acid potato-dextrose agar in petri-plates at 25°C. At the end of seven days

after inoculation the diameters of the developing colonies were measured. (Table 15).

Table 15.—The diameter growth in mm. of isolates 192 and 303 grown at 25°C on potato-dextrose agar buffered at various pH.

Isolate No.	Plate No.	pH 4.8	5.2	6.3	7.0	7.6	8.2	8.6
192	1	28.0	43.0	30.0	14.0	9.0	3.0[a]	3.0[a]
	2	28.0	47.0	25.0	25.0	14.0	3.0[a]	3.0[a]
	3	31.0	46.0	25.0	20.0	16.0	3.0[a]	3.0[a]
	4	19.0	46.0	25.0	20.0	9.0	3.0[a]	3.0[a]
	5	27.0	45.0	30.0	19.0	9.0	3.0[a]	3.0[a]
	average	26.6	45.4	27.0	19.6	11.4	3.0[a]	3.0[a]
303	1	3.0[a]	9.0	16.0	16.0	8.0	3.0[a]	3.0[a]
	2	3.0[a]	10.0	18.0	16.0	6.0	3.0[a]	3.0[a]
	3	3.0[a]	10.0	16.0	11.0	8.0	3.0[a]	3.0[a]
	4	3.0[a]	11.0	18.0	17.0	6.0	3.0[a]	3.0[a]
	5	3.0[a]	12.0	18.0	18.0	8.0	3.0[a]	3.0[a]
	average	3.0[a]	10.4	17.2	16.6	7.6	3.0[a]	3.0[a]

[a]Diameter of inoculum and actually represents no diameter growth.

From Table 15 it is evident that isolate 192 was capable of growing at a lower pH than isolate 303, since no measureable growth in diameter of the latter was detected at pH 4.8 while the growth of the former at the same pH was fairly good. How much greater an acid condition isolate 192 could withstand was not determined. Neither isolate grew under very high alkaline conditions; no measureable growth was detected at pH 8.2,

the critical pH being somewhere between pH 7.6 and pH 8.2. Of the pH values tested, the optimum growth of isolate 192 was at pH 5.2, and for isolate 303 between pH 6.3 to pH 7.0. It was also noted that at pH 4.8 the growth rate of isolate 192 was reduced to nearly half as compared to the rate of growth at optimum pH. The growth at pH 4.8 was greater than the growth at pH 7.0. Isolate 303 did not produce any noticeable growth at pH 4.8, but grew fairly well at pH 5.2, that is, considering the optimum rate of growth of this isolate.

Since isolate 192 grew best at a pH (5.2) lower than expected, both isolates were grown in liquid media of various pH values to check these results. One hundred and twenty-five ml. Erlenmeyer flasks were washed with sulfuric acid and potassium dichromate cleaning solution and washed three times with distilled water. At the same time sufficient quantities of minimal solution supplemented with yeast extract and casein were buffered at pH 4.2, 5.0, 5.9, 6.8, and 7.2. Twenty ml. of these media, respectively, were pipetted into the cleaned 125 ml. flasks, plugged and autoclaved at 15 pounds pressure for 20 minutes, after which the pH of each medium was taken again. In every instance the pH of the medium was not changed more than one tenth of a pH unit. The liquid of four flasks at each pH were seeded with loop transfers of conidial suspensions of isolate 192 and 303, respectively. These were placed at 27°C. The mycelial mats that developed on these media were washed, oven dried, and weighed. The growth of isolate 192 was allowed to proceed for nine days. Since isolate 303 grew much slower, it was allowed to grow for 18 days before drying. The results of this test are given in Table 16.

Table 16.--Growth of isolates 192 and 303 in liquid medium at various pH values.

Isolate No.	Flask No.	Weight in grams of mycelial mats at pH 4.2	5.0	5.9	6.8	7.2
192	1	.0337	.0472	.0553	.0703	.0498
	2	.0514	.0435	.0480	.0766	.0413
	3	.0496	.0392	.0405	.0714	.0466
	4	.0387	.0437	.0410	.0742	.0520
	average	.0433	.0434	.0462	.0731	.0474
303	1	.0000	.0024	.0228	.0540	.0000
	2	.0000	.0020	.0150	.0587	.0000
	3	.0000	.0018	.0168	.0510	.0000
	4	.0000	.0017	.0178	.0563	.0000
	average	.0000	.0020	.0181	.0550	.0000

The results of this test paralleled in most respects those of the previous one. The indications were that isolate 303 did not tolerate acidity as low as pH 4.2. Isolate 192 on the other hand produced a fair amount of growth in the medium of pH 4.2. The optimum growth for both isolates was at pH 6.8. While isolate 192 produced a fair growth at pH 7.2, isolate 303 failed to grow. There is no explanation for the fact that isolate 303 grew at pH 7.6 in the previous experiment, but not at pH 7.2 in this experiment. Aeration, however, might have had some influence on growth, since colonies grown in liquid media nearly always developed below the surface of the medium, while colonies grown on agar were restricted to the surface of the agar in a gaseous atmosphere.

From these pH studies of both isolates it was evident that isolates 192 and 303 responded differently to acid conditions, the latter being less adapted to the lower acid media tested than the former. The growth of isolate 303 was limited to a narrow range of pH, the limitations depending upon the type of medium. In liquid medium the range was probably pH 4.8 to pH 7.0. When the same isolate was grown on agar medium the range was about pH 5.0 to pH 8.0. Isolate 192 grew on all liquid media tested but not on agar at pH 8.2 or pH 8.6.

SEED TESTS FOR POSSIBLE INFECTION

Seeds of hairy vetch from a 1949-50 crop of commercial seed from Oregon were surface disinfested with clorox (1 part clorox to 4 parts distilled water) for five minutes in a sterile petri-plate. At the end of this soaking period the excess solution was drained off, and the seeds were plated out (5 seeds per plate) on non-acid potato-dextrose agar. Each seed was seated below the surface of the agar. These plates were stored at 72°F for 14 days and then examined for developing colonies of fungi. Of a total of 1,455 seeds plated 811 germinated, 1,405 remained sterile, and bacterial colonies developed from 50. No fungi developed.

Common vetch seeds were harvested on May 31, 1950 from a planting at the Louisiana State University Horticultural farm. The amount of seeds collected was very limited, since the common vetch plants in the field were severely infected with anthracnose diseases and as a result produced very few seeds. Some of the seeds obtained were of very poor quality and already partly covered with mycelium of fungi. These seeds were dried at room temperatures and stored until October 23, 1950, at which time they were surface disinfested with diluted clorox and plated out on potato-dextrose agar, deeply enough so that the seeds were seated below the surface of the agar. The plates were stored at 72°F for 14 days when they were observed for seed-borne infection caused by Colletotrichum spp. From a total of 941 seeds plated, 632 germinated, 338 remained sterile, bacterial colonies developed from 229, and from the remainder, Fusarium spp. developed from 65, Aspergillus sp. from 10, Gloeosporium sp. from 3, Alternaria sp. from 1, and Penicillium sp. from 1. None of the Colletotrichum spp. were isolated from these seeds.

Another group of commercial hairy and common vetch seeds were surface disinfested with clorox and spread over moist filter paper in large moist chambers in the laboratory. Most of these seeds germinated within several days. The seedlings were observed daily for two weeks in order to attempt to detect any Colletotrichum spp. that might have developed from them. Of a total of 3,641 hairy vetch seeds and 2,912 common vetch seeds Colletotrichum spp. developed from none. Common fungus saprophytes and a few bacterial colonies were present on both common and hairy vetch seed coats and some of the injured cotyledonous tissue. Transplants were made to agar from spots that developed on the basal stems of 25 seedlings, although these spots were not typical of those produced by isolates 192 or 303. No fungi grew from these plant tissue cultures within eight days.

SPECIFIC RESISTANCE

Preliminary inoculation studies were conducted with common and hairy vetch, the two principal commercial vetch species used as cover crops in Louisiana. A group of five isolates which produced "straight" conidia and which were isolated from field peas and vetch were tested on common and hairy vetch. The plants were grown in steamed soil in six-inch pots in the greenhouse. They were atomized with heavy conidial suspensions of the test isolates, and placed in the moisture chamber for several days. Within four days, spots appeared on both common and hairy vetch plants. A week after inoculation the common vetch plants were severely defoliated and a number were dead. The hairy vetch plants, even though they exhibited numerous leaf and stem spots, were not severely defoliated or killed. Additional inoculations with the same isolates were made on common and hairy vetch. The same results were obtained; that is, common vetch was very susceptible and hairy vetch was somewhat resistant.

Another group of five isolates which produced curved conidia and which were isolated from vetch, were tested for pathogenicity on common and hairy vetch. The plants were grown in steamed soil in pots in the greenhouse. They were inoculated with conidial suspensions from these isolates, respectivsly, and placed in the moisture chamber for several days. Within four days lesions appeared on the inoculated plants. A week after inoculation the hairy vetch plants were severely infected, but the common vetch plants showed considerable resistance.

From these inoculation tests it was evident that common vetch was susceptible to isolate 192, and resistant to isolate 303. On the other hand, hairy vetch was susceptible to isolate 303 and resistant to isolate 192.

In additional tests a large number of seed lots with plant introduction and forage crop numbers were tested with the representative isolates, 192 and 303. These included the following vetch species: Vicia sativa, V. villosa, V. dasycarpa, V. alba, V. angustifolia, V. pannonica, V. articulata (V. monantha), V. atropurpurea, and V. grandiflora. Other species tested were V. ludoviciana, V. hirsuta, and V. aurantia. These varieties of vetch were grown in sterilized soil in three-inch pots in the greenhouse, and inoculated with conidial suspensions of isolates 192 and 303, respectively. The plants were placed in the moisture chamber for 72 hours and then removed to a greenhouse bench. Each time inoculations were made approximately 20 plants of each selection were inoculated (two pots of each with 10 plants per pot). Controls consisted of uninoculated plants placed in the moisture chamber with the inoculated ones. Some selections of vetch were tested as many as 7 times, others only once, since in some instances just a few seeds were available. The results of inoculations over a two-year period are tabulated in Table 17.

Inoculations were rated by the following system: VR, slight infection, only a few spots on several plants; R, mild infection, a few spots on a large number of plants; MR, medium infection, appreciable number of spots on a large number of plants but no killing; S, severe infection, numerous spots on all plants and killing of some plants; and VS, very severe infection, killing of a large number of plants.

From these tests the following vetch species were considered to be resistant to isolate 192: V. atropurpurea, V. ludoviciana, and V. villosa. The first two species were more resistant than the latter. V. villosa developed an appreciable number of spots on a large number of

Table 17.--Resistance or susceptibility of varieties of vetch to isolates 192 and 303 tested under greenhouse conditions during 1949, 1950, and 1951.

Seed lot No.	Variety or species name and F.C. or other number	Isolate No. 192	No. times tested	Isolate No. 303	No. times tested
1	F.C. 23,709 *Vicia sativa*	VS	5	R	3
2	P.I. 117,425 *V. sativa*	S	2		0
3	_____ *V. sativa*, light mottled seed	VS	2		0
4	F.C. 29,993 *V. sativa*	S	6	R	5
5	F.C. 18,808-3 *V. sativa*	S	2		0
6	F.C. 18,808-1 *V. sativa*	S	2		0
7	P.I. 143,511 *V. sativa*	VS	2		0
8	_____ *V. sativa*, Oregon common goldiron	S	3	VR	2
9	F.C. 31,542 *V. sativa*	S	3	R	1
10	F.C. 16,462 *V. sativa*	S	3	R	2
11	F.C. 18,818 *V. sativa*	S	2		0
12	_____ *V. sativa*, Willamette	VS	5	R	2
13	_____ *V. sativa*, Willamette, dark mottled seed	VS	2		0
14	F.C. 23,723 *V. sativa*, Willamette	VS	3	R	2
15	_____ *V. sativa*, Oregon	VS	5	R	4
16	_____ *V. sativa*, Sweeden	VS	3	VR	1
17	_____ *V. alba*	S	2		0

Table 17 (Cont'd).

Seed lot No.	Variety or species name and F.C. or other number	Isolate No. 192	No. times tested	Isolate No. 303	No. times tested
18	F.C. 2,830 V. alba	VS	1		0
19	F.C. 23,753 V. alba	VS	3	R	3
20	_____ V. villosa, Oregon	MR	6	S	5
21	_____ V. villosa, Texas	MR	3	S	1
22	F.C. 23,724 V. villosa	MR	4	S	4
23	F.C. 23,404 V. villosa	MR	2	S	3
24	F.C. 18,139 V. monantha (V. articulata)	VS	5	S	3
25	F.C. 31,544 V. monantha (V. articulata)	S	2		0
26	_____ V. pannonica, light seeded	S	3	R	2
27	_____ V. pannonica	S	3	MR	2
28	F.C. 23,763 V. pannonica	VS	4	R	4
29	F.C. 23,755 V. pannonica	VS	3	R	3
30	F.C. 30,650 V. atropurpurea	VR	3	S	2
31	_____ V. atropurpurea	VR	3	S	2
32	F.C. 31,049 V. atropurpurea	R	3	S	2
33	F.C. 23,757 V. atropurpurea	R	2	S	2
34	F.C. 23,764 V. dasycarpa	VS	6	S	7
35	F.C. 23,761 V. dasycarpa	S	4	S	4
36	F.C. 31,771 V. grandiflora	VS	3	MR	2
37	F.C. 31,898 V. grandiflora	VS	6	MR	7

Table 17 (Cont'd).

Seed lot No.	Variety or species name and P.C. or other number	Isolate No. 192	No. times tested	Isolate No. 303	No. times tested
38	_____ V. angustifolia	S	1	R	1
39	_____ V. angustifolia	VS	2	R	3
40	P.C. 23,994 V. angustifolia	VS	1	R	2
41	_____ V. ludoviciana	R	2	MR	3
42	_____ V. hirsuta	S	1	R	2
43	P.I. 190,252 V. aurantia	S	1	R	1

plants when inoculated with isolate 192, but none of the plants were killed. When the other two species were inoculated with isolate 192, only a few spots developed on several plants, or in some instances, a few spots were formed on many of the plants. Vicia sativa, V. alba, V. dasycarpa, V. angustifolia, V. pannonica, V. grandiflora, V. articulata, V. hirsuta, and V. aurantia were considered to be susceptible to isolate 192. When these species were inoculated with isolate 192, severe or very severe infection resulted. In all cases numerous spots developed on the leaves and stems. The spots often coalesced so that the stems and petioles were girdled, causing them to be greatly weakened at these points, bend over, and soon die. The killing of susceptible plants was aided by rapid defoliation. Infection was so severe that many plants were killed in seven days after inoculation.

All of the vetch species inoculated with isolate 192 were also tested with isolate 303 in the greenhouse, although not all of the seed lots were included. The following species of vetch were considered to be resistant to isolate 303: V. alba, V. sativa, V. aurantia, V. pannonica,

V. angustifolia, *V. grandiflora*, and *V. hirsuta*. The species *V. atropurpurea*, *V. villosa*, *V. dasycarpa*, *V. ludoviciana*, and *V. articulata* were considered susceptible.

It was important to note that most of the vetch species that were susceptible to isolate 192 were resistant to isolate 303. Most of the species resistant to isolate 192 were susceptible to isolate 303. Only two cases were the exception: *V. dasycarpa* and *V. articulata* were susceptible to both isolates.

HOST RANGE

Since some of the original isolates representing the 192 type Colletotrichum were obtained from field peas, similar greenhouse grown plants were inoculated with this isolate. They included Creole, Dixie Wonder, and Austrian Winter peas. The plants were grown in steamed soil in three-inch pots (ten plants per pot), inoculated with conidial suspensions, and placed in the moisture chamber for three days. Within four days spots appeared on a large number of the plants, particularly on the leaves. Individual leaf spots were several mm. in diameter, often coalescing to involve the entire leaf. Stem spots occurred much less frequently, but sometimes stems were girdled where several spots coalesced. A description of spots on field peas is given in the next section.

Since peas were susceptible upon inoculation, a number of other plants were grown in the greenhouse and tested with isolates 192 and 303. Included in this group were red clover (Trifolium pratense, L.), soybeans (Soja Max, Piper) Oklahoma 710, golden gram (Phaseolus aureus, Roxb.) P. I. 164,770, common alfalfa (Medicago sativa, L.), Sericea lespedeza (Lespedeza sericea, Don.), Kobe lespedeza (L. striata, Hook and Arn.), Singletary peas (Lathyrus hirsutum, L.), Lotus uliginosus, sweet peas (L. odoratus, L.), Dixie Wonder peas (Pisum arvense, L.), Austrian Winter peas (P. sativum var. arvense, L.), and Creole peas (P. sativum, L.). These plants were inoculated in the usual manner with isolates 192 and 303, placed in the moisture chamber for three days and then removed to a greenhouse bench. Spots appeared on leaves and stems of the Dixie Wonder, Austrian Winter, Creole, Singletary, and sweet peas

within four days after inoculation with isolate 192. Four days later the leaves of these same plants had become severely infected. Infection was not severe on the stems, but some plants were girdled and killed. However, the leaves showed many spots. The older leaves of the Dixie Wonder, Austrian Winter, and Creole peas were frequently chlorotic around the spots. This symptom was rarely observed on younger leaves. Leaf spots on the Singletary and sweet peas were somewhat different in appearance as described in the next section; the areas surrounding them did not become chlorotic.

Plants susceptible to isolate 192 were resistant to isolate 303. Only a few spots were observed on the Creole, Dixie Wonder, and Austrian Winter peas. Such spots were nearly always formed on senescent leaves. They appeared 10 to 14 days after inoculation. No stem infection was noticed. Very few spots were observed on stems and leaves of Singletary and sweet peas. Again infection was noticed 10 to 14 days after inoculation.

Another series of greenhouse grown plants were inoculated with isolates 192 and 303, including Zenith rice (Oryza sativa, L.), oats (Avena sativa, L.), corn (Zea Mays, L.), Singletary peas (Lathyrus hirsutum, L.), blue lupine (Lupinus hirsutus, L.), white lupine (L. albus, L.), yellow lupine (L. luteus, L.), Kobe lespedeza (Lespedeza striata, Hook and Arn.), Korean lespedeza (L. stipulacea, Maxim.), crimson clover (Trifolium incarnatum, L.), and Melilotus indica, All. Singletary peas and M. indica were susceptible to isolate 192. Isolate 303 produced spots on the latter, but infection was not severe. The symptoms produced by isolates 192 and 303 on Singletary peas are described in the next

section. Isolate 192 caused severe infection on the petioles of M. indica but only a few spots were produced on the leaves. Petioles were frequently girdled, killing the leaves of many of the plants eight days after inoculation.

Iron cow peas (Vigna sinensis, Endl.), black eyed peas (V. sinensis, Endl.), Valencia peanuts (Arachis hypogaea, L.), Spanish peanuts (A. hypogaea, L.), Kansas Grimm alfalfa (Medicago sativa, L.), white clover (Trifolium repens, L.), Persian clover (T. resupinatrum, L.), white dutch clover (T. repens, L.), alyce clover (Alysicarpus vaginalis, D. C.), Davidson barley (Hordeum vulgare, L.), Crotalaria sp. L., C. spectabilis, and C. intermedia were also inoculated with isolates 192 and 303. The inoculation was carried out as in previous tests. None of the plants became infected.

SYMPTOMS ON ARTIFICIALLY INOCULATED PLANTS

On vetch plants:

The spots produced by isolate 192 on all species of vetch tested in the greenhouse were similar. Those caused by isolate 303 varied somewhat depending on the vetch species being attacked. The response of greenhouse inoculated plants with isolate 192 will be considered first.

Individually isolated leaf spots caused by isolate 192 were circular to elliptical. Frequently, spots coalesced so that nearly half a leaflet or sometimes an entire leaflet was involved. In such cases the necrotic areas were irregular in shape. The isolated spots varied in size up to three mm. in diameter but commonly were one to two mm. in diameter. The spots were noticeable within four days after inoculation, depending on the variety attacked. They appeared within four days on the susceptible varieties and within five days on the more resistant varieties. The first evidence of infection occurred as water-soaked spots, lighter green than the normal leaf tissue surrounding it, and up to three mm. in diameter. This suggested that the spots probably had reached their maximum size at this stage in their development. Within another 24 to 48 hours the spots lost their green pigment, usually becoming cinnamon-brown, surrounded by a narrow garnet-brown of Ridgway border. The brown center often turned nearly white as the lesions aged. Sometimes groups of acervuli and/or black sclerotial masses developed in these spots, appearing as small black dots in the center of the spots, particularly when subjected to moist conditions. At this stage of

infection defoliation was severe on the susceptible varieties. The leaflets became yellow and finally dropped. In case of very heavy infection leaflets dropped much earlier in the development of the disease, even before the spots had lost completely their green pigment. Spots occurred on both sides of the leaflets but were usually found on the upper surfaces.

The stem and petiole spots at first were sunken, light green, and usually several mm. long, but up to four mm. and about one mm. wide. Some spots appeared larger but this was caused by spots coalescing. Stems and petioles were often girdled. Within 48 hours these spots darkened, sometimes becoming similar to those found on leaves. The size of the spots usually depended on the stem diameter of the host. Spots were larger on common vetch stems than on hairy vetch stems because of the difference in stem thickness. Often the stem spots appeared as dark brown to blackened, slightly sunken streaks smaller than the previously described ones, particularly on hairy vetch. Under moist conditions acervuli developed on infected stem tissue. Stems of nearly mature plants in the field became blackened half the distance up the stem. Greenhouse inoculated plants were never maintained long enough for this symptom to occur.

Spots produced by isolate 303 on susceptible species of vetch were similar to those produced by isolate 192. However, on resistant varieties the spots were mostly fleck-like, first reddish-brown in color, then became blackened. These fleck-like spots were irregular in shape, less than one mm. in diameter on leaves, but somewhat elongated on the stems. Under moist conditions they produced black, sclerotial masses. Otherwise the spots formed by both isolates appeared similar. Photographs

of stem and leaf spots appear on Plates III and V.

On Austrian Winter, Dixie Wonder, and Creole peas:

Leaf spots produced by isolate 192 were nearly circular with almost regular margins, except when the infection followed the veins a short distance from the edge of the spot. The spots appeared as water-soaked areas at first, but later became dark green until they were olive-green of Ridgway with a much darker green, narrow border. Individual spots ranged from 0.5 mm. to 2.5 mm. in diameter. Chlorosis about the spots was often associated with older leaves and not usually with spots on younger leaves. Spots on the older chlorotic leaves appeared almost as green as the normal green of healthy leaves. Spots on young leaves caused the spots to appear olive-green to grayish-green in color.

Stem spots were elliptical in shape, sunken and three to four mm. long and one to two mm. wide. At first they appeared as water-soaked areas, later turning light-brown, and surrounded by a narrow dark-brown border.

Spots produced by isolate 303 were observed on senescent leaves only. They appeared about 12 to 14 days after inoculation. These spots were similar to those produced by isolate 192. Photographs of stem and leaf spots are shown on Plates IV and V.

On sweet and Singletary peas:

On these plants isolate 192 produced somewhat different spots from those already described. They first appeared as water-soaked areas on the leaves. As the spots became older, they turned nearly white and were uniformly colored. Spots were round to oval and up to two mm. in diameter. Stem spots were similar in color to those on the leaves but

different in shape. They were up to two mm. wide and four mm. long.

Isolate 303 produced very few spots on either sweet or Singletary peas within 12 days after inoculation. They were similar to those produced by isolate 192.

On Melilotus indica:

Few spots occurred on the leaves when inoculated with isolate 192. Those present were irregular in shape, in many cases angular, light-brown throughout, and sunken. More important were the numerous, small, elongated, coalescing spots less than 0.5 mm. in diameter on the petioles. Many petioles were girdled, reducing the foliage considerably.

This infected tissue first resembled water-soaked areas, but soon turned light tan in color.

Isolate 303 did not produce any noticeable spots on M. indica.

TECHNICAL DESCRIPTION OF ISOLATE 192

Leaf spots typically circular to linear, sometimes irregular, light brown to gray with garnet-brown to warm sepia, narrow border. Stem spots similar in color, becoming dark brown to black, linear in shape. On stems acervuli 55 to 125 u in diameter and not confluent, on leaflets amphigenous or hypophyllus. Conidial masses ochraceous-buff to gray. Setae wanting or abundant, Brussel's-brown at the base, becoming lighter brown to gray at the tips, average 126.9 u (range 60 to 200 u) long by 7.1 u (range 6.5 to 9.3 u) wide across the bulbous base, tapering to a rounded or sometimes nearly pointed tip, containing 1 to 5 septa. Conidia hyaline, nearly straight, bluntly tapered, unicellular, average 21.2 u (range 16.7 to 25.9 u) by 3.8 u (range 3.7 to 5.6 u), the most common size 22.2 u by 3.7 u.

DISCUSSION

Weiss (33) lists the following anthracnose fungi as having been reported on Vicia spp., excluding V. faba, in the United States: Colletotrichum viciae, C. villosum, Kabatiella nigricans, and Gloeosporium davisii. Of the isolations made by the writer in 1948, 1949, and 1950, C. villosum was the only Colletotrichum species of this group that was isolated from diseased vetch plants in Louisiana. Bain (3, 4, 5) reported C. viciae on vetch in Mississippi and Louisiana in 1944, but it was pointed out later (19) that the fungus observed was C. villosum. C. viciae was listed by Weiss (33) to occur in Louisiana and Mississippi. These reports were probably from Bain's observations in 1944 and 1945. Therefore, it was obvious that C. viciae was not found in Louisiana as indicated at that time. Since the conidia of C. villosum and C. viciae are similar in shape, and since no other Colletotrichum species on vetch had been described at the time of Bain's surveys, the confusion is easily understandable.

C. villosum (isolate 303 type) was described by Weimer (29) in 1945. His publication pointed out that hairy vetch was susceptible to this fungus, whereas common vetch was resistant. A previous survey (28) indicated similar observations on field-grown plants. Reports by the Georgia Experiment Station (31, 32) for the following three years indicated that the common vetch plants were severely infected with anthracnose. The implication was that the same fungus was involved. From inoculation studies given in this paper and Weimer's inoculation tests it was shown that common vetch was resistant to C. villosum so

that another Colletotrichum species must have been the cause for the sudden susceptibility of common vetch. From all indications the "straight" conidial type was responsible for this phenomenon and became established in the Georgia Experiment Station area later than 1945, after Colletotrichum villosum had been described. Since the symptoms caused by both organisms were similar, field examination alone was not adequate to indicate that two species were involved in the disease complex.

No experimental evidence was obtained to determine how the "straight" conidial type became established. It is likely, however, that it was introduced by infected seeds. A large number of commercially grown seeds of common and hairy vetch were germinated in moisture chambers and in agar in the laboratory. Common vetch seeds obtained from severely infected pods were also germinated in agar. No Colletotrichum spp. were observed to be present on the developing seedlings. Weimer (29) mentioned that the use of clean, diseased-free seed is a possible means of control for C. villosum. But he gave no experimental evidence that vetch seeds carry the fungus. Diseased plant material, which was stored in wire baskets outside the greenhouse over the summer, was also placed in moisture chambers in the laboratory. No anthracnose fungi were observed to develop from this plant material. Since field peas were found to be susceptible to the 192 type, and since mixtures of field peas and vetch were frequently used as a winter cover crop on some of the farms visited in Louisiana, the disease problem becomes more complicated. Native vetch species were found to be susceptible to the "straight" conidial type. Such plants possibly are responsible for a continued source of inoculum.

Twelve species of vetch were used in inoculation tests in the greenhouse. Only three were considered resistant to isolate 192. Seven were considered resistant to isolate 303. With two exceptions those species that were susceptible to isolate 192 were resistant to isolate 303 and vice versa; Vicia dasycarpa and V. articulata (V. monantha) were susceptible to both isolates. Weimer (29) indicated that V. articulata was resistant to Colletotrichum villosum. All other species of vetch tested with C. villosum were in agreement with those tested by Weimer.

The resistant varieties recovered within several weeks after inoculation when they were removed from the highly humid conditions, that is, removed from the moisture chamber and placed on a greenhouse bench. Most of the infected leaves dropped off, but defoliation was not severe. Many of the resistant varieties proceeded to grow normally after the loss of the infected leaves. In order for new or continued infection to occur, the plants would have had to be placed in a highly humid atmosphere with a means of disseminating the conidia. A similar condition was observed in the field. Plants which were growing on dead cotton stalks were usually less infected than plants which were not given support. Such vetch plants, no doubt, were raised sufficiently so that the lower stems and leaves were not as continually subjected to a highly humid condition. The most susceptible varieties were usually killed or so badly damaged that the plants did not recover, even though they were removed from the moisture chamber free of excess humidity.

SUMMARY

1. Four different anthracnose fungi were found associated with field-grown, diseased vetch plants in Louisiana. The Colletotrichum spp. which produced either curved or "straight" conidia were the only ones which were pathogenic to vetch, according to greenhouse inoculation tests.
2. The fungi pathogenic to vetch species were C. villosum and isolate 192 type. A technical description of the latter was given.
3. A detailed study was made of C. villosum (isolate 303) and isolate 192. They were found to differ in morphological, physiological, and cultural characteristics.
4. Vicia atropurpurea, V. ludoviciana, V. villosa were resistant to the isolate 192 type; V. sativa, V. dasycarpa, V. angustifolia, V. pannonica, V. grandiflora, V. articulata, V. hirsuta, V. alba, and V. aurantia were susceptible. V. alba, V. sativa, V. angustifolia, V. pannonica, V. grandiflora, V. aurantia, and V. hirsuta were resistant to C. villosum. V. atropurpurea, V. villosa, V. dasycarpa, V. ludoviciana, and V. articulata were susceptible.
5. Austrian Winter, Dixie Wonder, Creole, sweet, and Singletary peas were susceptible to isolate 192 type. Similar plants were resistant to C. villosum. The former also attacked Melilotus indica while the latter did not.
6. Neither C. villosum nor isolate 192 type were found to develop from common and hairy vetch seedlings grown on moist filter paper or in potato-dextrose agar within two weeks after germination.

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VITA

Norman Louis Horn, Jr. was born August 9, 1919, in Lewistown, Pennsylvania. In 1923 he moved to Gettysburg, Pennsylvania, and attended elementary school from 1925 to 1929. In 1929 he moved to Baltimore, Maryland, where he enrolled in the public school. He entered junior high school in 1932 and from 1935 to 1937 attended the high school of Baltimore City College. In 1939 he entered the University of Maryland, College Park, Maryland and completed the requirements for the B. S. degree with a major in Botany. He accepted a position of scientific aid at the Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Maryland, in 1944. Here he worked until February, 1946, with Dr. R. C. Thompson, Agronomist. In February, 1946, he again enrolled at the University of Maryland as a graduate student, and at the same time worked with Dr. W. D. McClellan, Pathologist, at the Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland. In September, 1946, he accepted an appointment as graduate assistant in the Department of Botany at the University of Maryland. Here he taught Botany laboratory and took course work until he received his M. S. degree in Botany in June, 1948. He obtained a fellowship in the Department of Botany, Bacteriology, and Plant Pathology at Louisiana State University, Baton Rouge, Louisiana, in September, 1948. During the spring of 1951 he completed his requirements for the degree of Doctor of Philosophy in Plant Pathology at this University.

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LEGEND FOR PLATES

PLATE I

Fig. 1. Natural leaf spot infection caused by Colletotrichum villosum (isolate 303) and Colletotrichum sp. (isolate 192), common vetch leaflets above and hairy vetch leaflets below.

Fig. 2. This photograph was taken on March 24, 1950, showing that common vetch (left) was killed chiefly by anthracnose, while hairy vetch (right) retained good growth.

PLATE II

Stem and pod spot on common vetch (above) and hairy vetch (below) caused by Colletotrichum villosum (isolate 303) and Colletotrichum sp. (isolate 192 type). Natural infection.

PLATE III

Fig. 1. Leaf spot of hairy vetch (left) and common vetch (right). The leaflets above were inoculated with Colletotrichum villosum (isolate 303), those below with Colletotrichum sp. (isolate 192).

Fig. 2. Leaf spot of common vetch inoculated with Colletotrichum villosum (isolate 303).

PLATE IV

Fig. 1. Leaflets above were from plants 7 days after they were inoculated with Colletotrichum sp. (isolate 192). Leaflets below were from plants not inoculated. Leaflets in groups of four from left to right were from Dixie Wonder, Creole, and Austrian Winter pea plants.

Fig. 2. Common vetch plants on the right 7 days after they were inoculated with Colletotrichum sp. (isolate 192). Plants on the left were not inoculated.

PLATE V

Fig. 1. Stem spots on domestic common vetch (left) and imported common vetch (right) caused by Colletotrichum sp. (isolate 192).

Fig. 2. Stem spots on Austrian winter peas (left) and Dixie Wonder peas (right) caused by Colletotrichum sp. (isolate 192).

Fig. 3. Stem spots on common vetch caused by Colletotrichum villosum (isolate 303).

PLATE VI

Fig. 1. Basal stem infection on vetch caused by Colletotrichum sp. (isolate 192). The plant parts in groups of four from left to right are common vetch grown in infested soil, common vetch controls, hairy vetch grown in infested soil, and hairy vetch controls.

Fig. 2. Basal stem infection on vetch caused by Colletotrichum villosum (isolate 303). The plant parts in groups of four from left to right are common vetch grown in infested soil, common vetch controls, hairy vetch grown in infested soil, and hairy vetch controls.

PLATE VII

Fig. 1. Colletotrichum sp. (isolate 192) was grown at 27°C for seven days on potato-dextrose agar to which was added 0 (top left), 1 (top right), 2 (bottom left), and 3 (bottom right) drops of 50 per cent lactic acid per 15 ml. of medium.

Fig. 2. The growth rate of Colletotrichum villosum (isolate 303) was compared with Colletotrichum sp. (isolate 192) in Fries liquid medium supplemented with yeast extract at 27°C and buffered at pH 5.5.

PLATE VIII

Fig. 1. Colletotrichum sp. (isolate 192). A, B, C, D, E, F, and H are various stages of growth from a conidial suspension in Fries liquid medium supplemented with yeast extract; E, demonstrates the means of conidial production in the same medium; G, appresoria were produced abundantly when conidia were germinated in sterilized, distilled water; I, are conidia.

Fig. 2. Colletotrichum villosum (isolate 303). B, C, D, F, and H are various stages of growth from a conidial suspension of Fries medium supplemented with yeast extract; A, represents the formation of appresoria which were produced abundantly when conidia germinated in sterilized, distilled water; E, is a hyphal tip developing from an appresorium; G, are conidia.

PLATE IX

Fig. 1. Colletotrichum villosum (isolate 303) left, Colletotrichum sp. (isolate 192) center, and Colletotrichum sp. (isolate 192-Y) right, grown on potato-dextrose agar for eight days at 27°C.

Fig. 2. Conidia of Colletotrichum villosum (isolate 303) from potato-dextrose agar.

Fig. 3. Conidia of Colletotrichum sp. (isolate 192) from potato-dextrose agar.

PLATE X

Fig. 1. An acervulus developing from stem tissue of a greenhouse-grown, common vetch plant inoculated with Colletotrichum sp. (isolate 192).

Fig. 2. An acervulus developing from stem tissue of a greenhouse-grown, common vetch plant inoculated with Colletotrichum villosum (isolate 303).

PLATE I

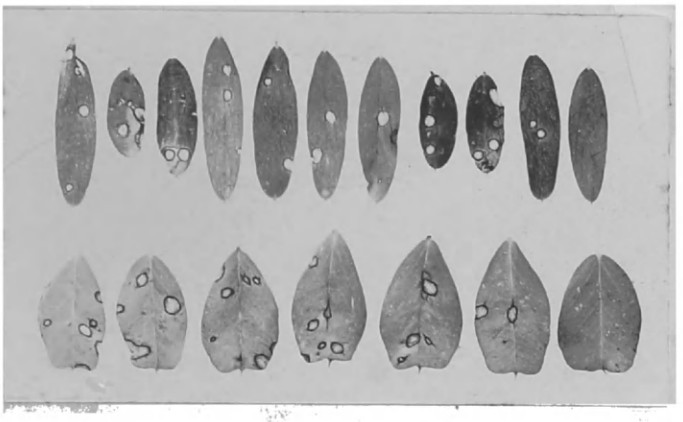


Fig. 1.

Fig. 2.

PLATE II

PLATE III

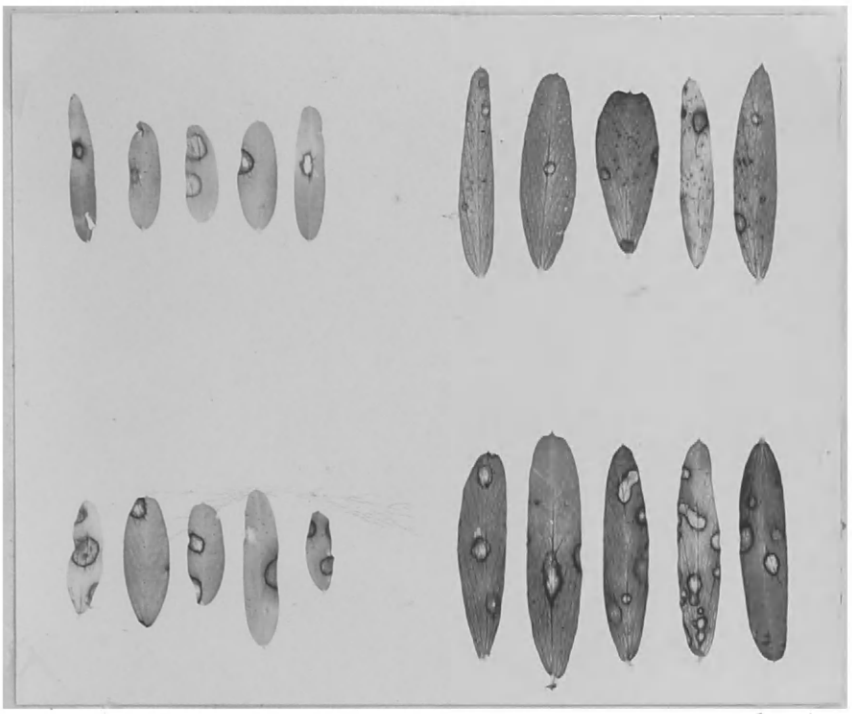


Fig. 1.

Fig. 2.

PLATE IV

Fig. 1.

Fig. 2.

PLATE V

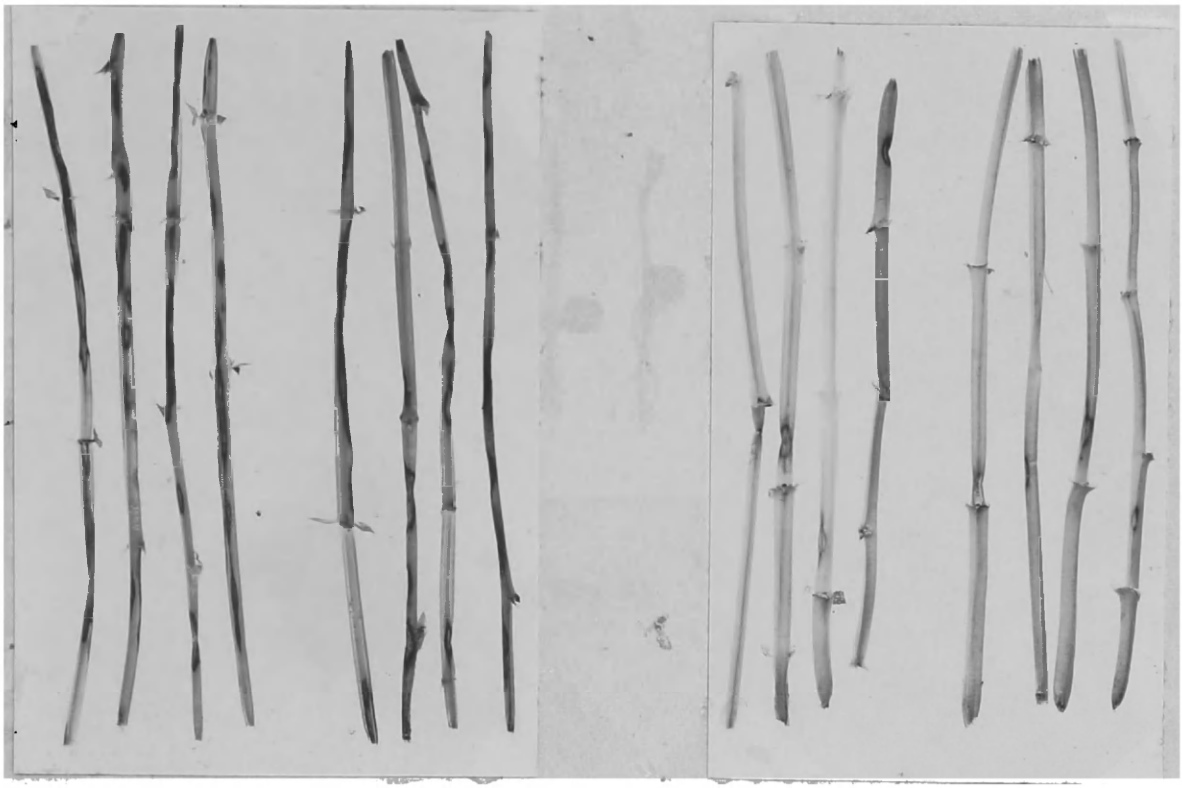


Fig. 1.

Fig. 2.

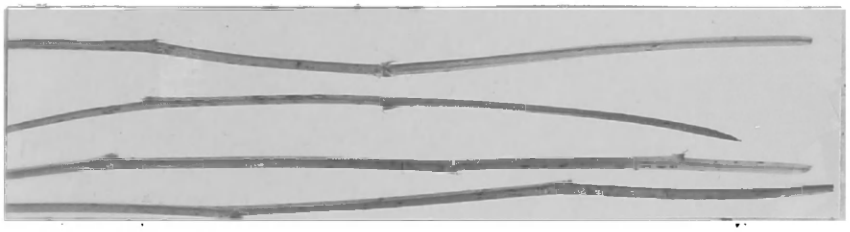
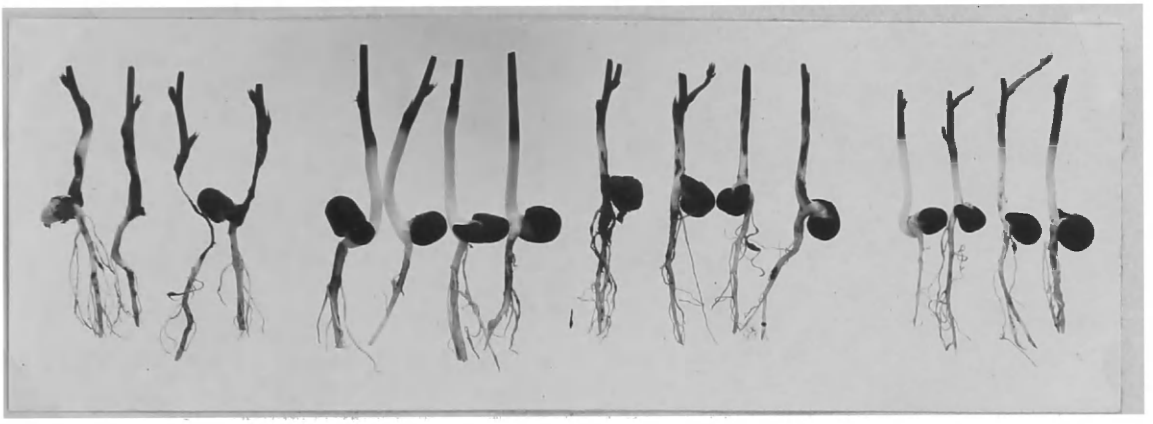


Fig. 3.

PLATE VI



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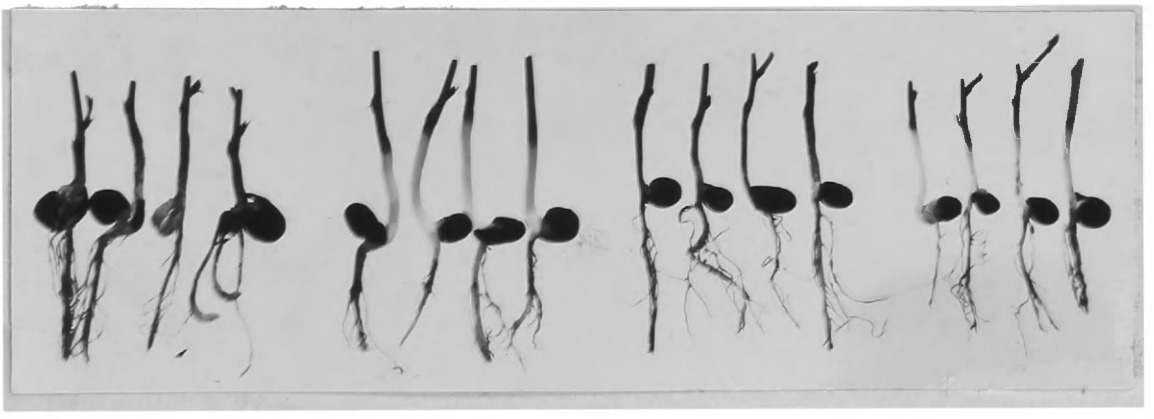
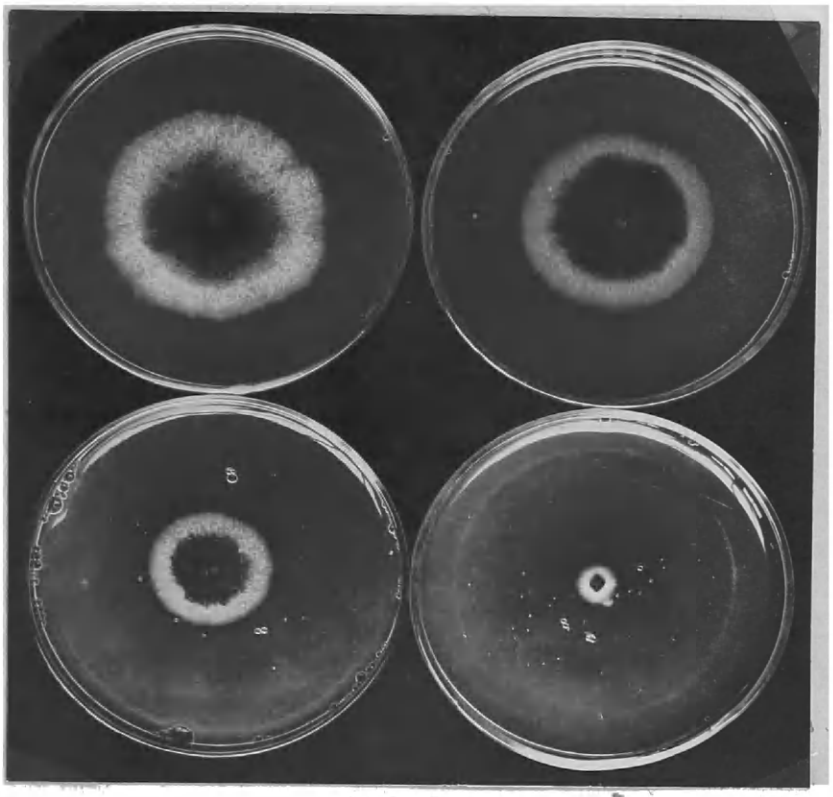


Fig. 2.

PLATE VII



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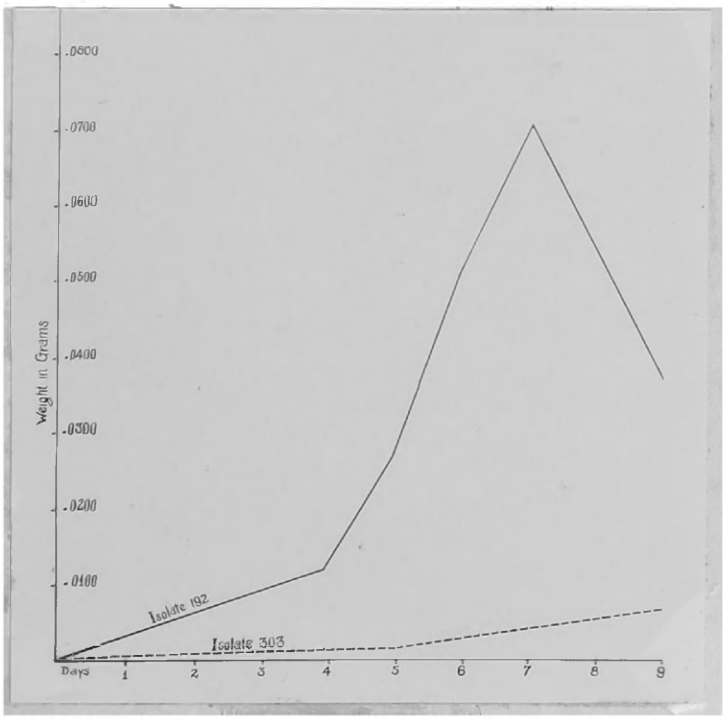
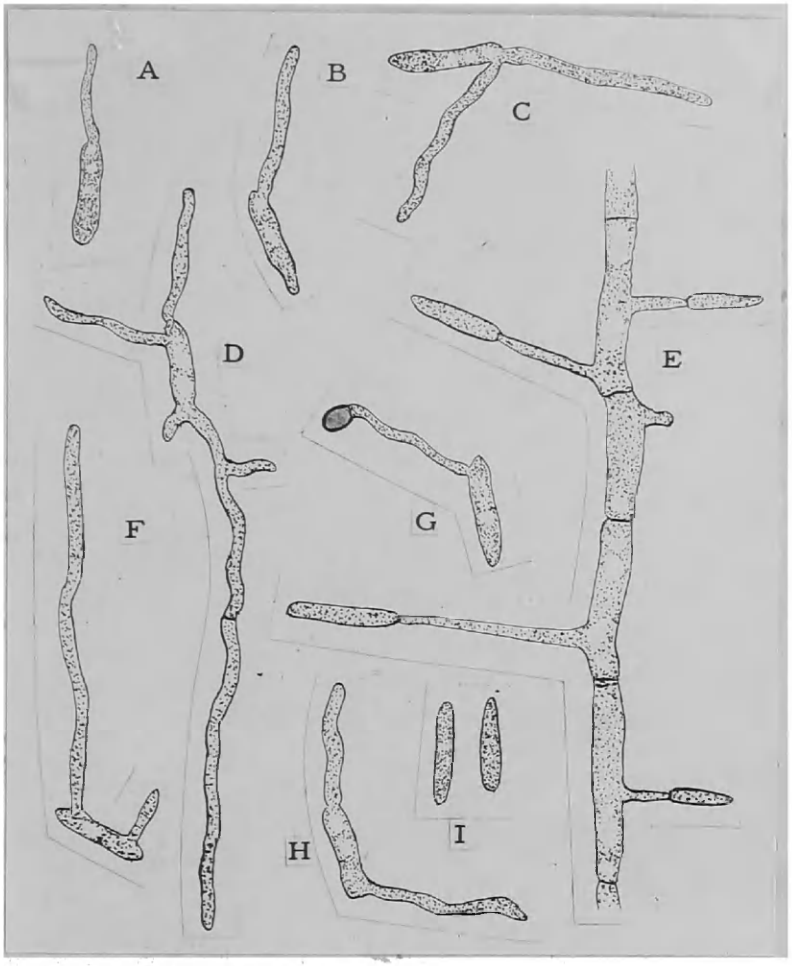


Fig. 2.

PLATE VIII



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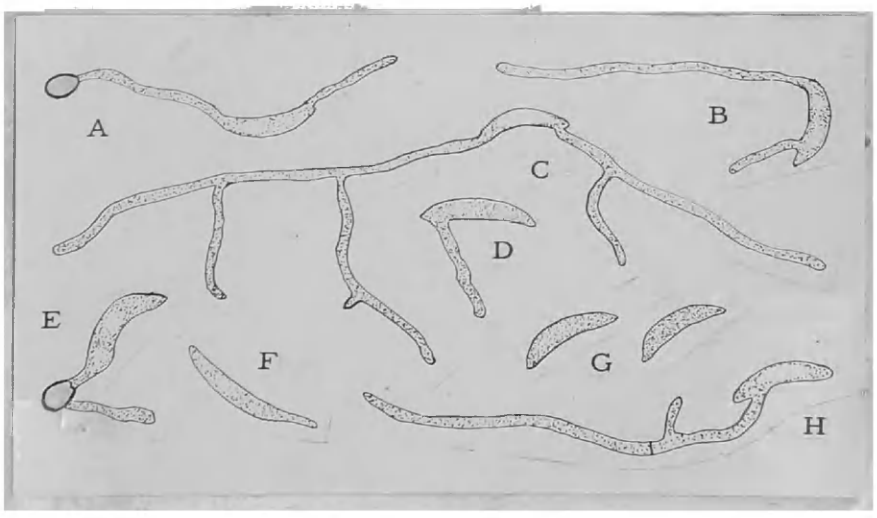

Fig. 2.

PLATE IX

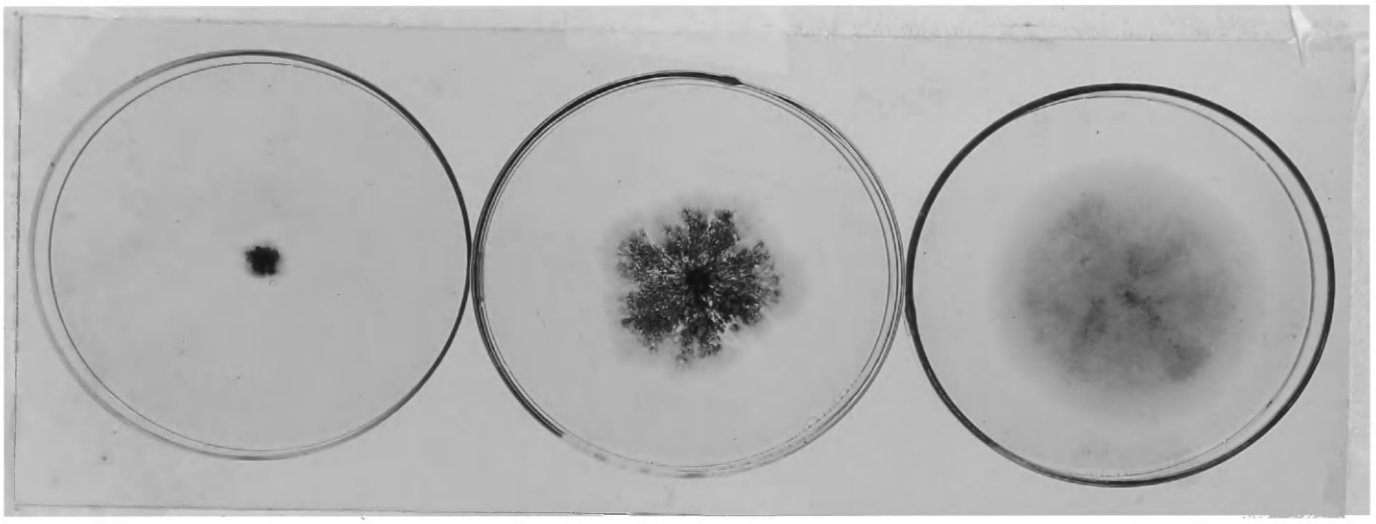

Fig. 1.

Fig. 2.

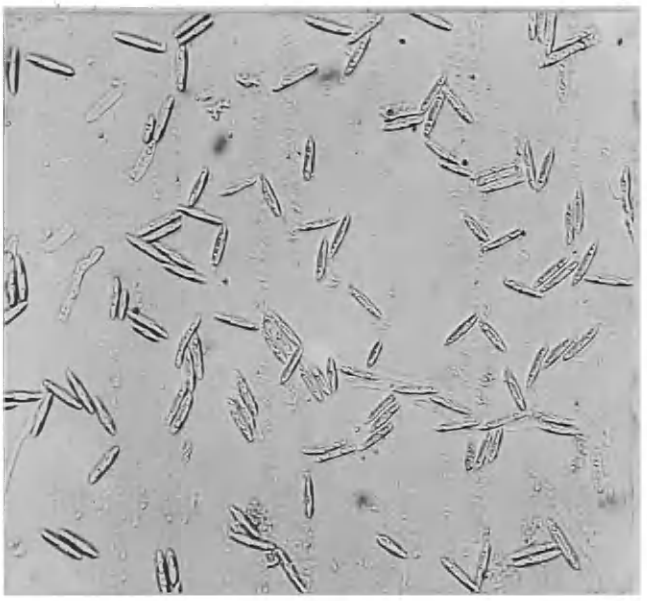

Fig. 3.

PLATE X

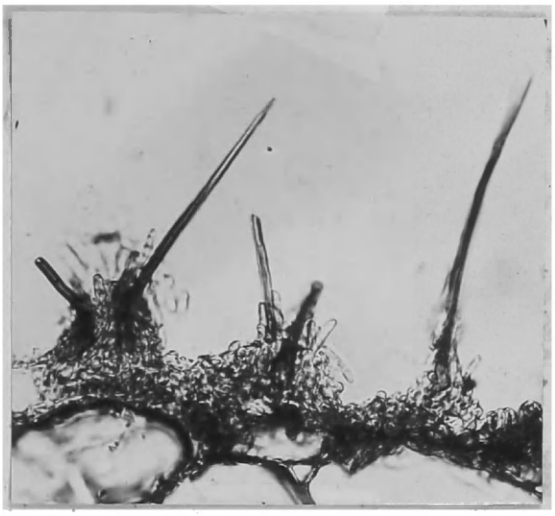

Fig. 1.

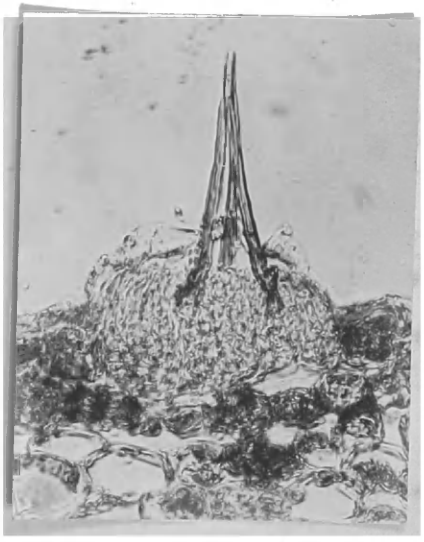

Fig. 2.

EXAMINATION AND THESIS REPORT

Candidate: **Norman Louis Horn, Jr.**

Major Field: **Plant Pathology**

Title of Thesis: **Studies of Vetch Anthracnoses**

Approved:

St. John P. Chilton
Major Professor and Chairman

Richard H. Russell
Dean of the Graduate School

EXAMINING COMMITTEE:

O. W. Edgerton

A. S. Blakeslee

H. C. Thurlow

M. T. Henderson

J. D. Atkins

L. H. Flint

Date of Examination:

May 2 1951